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A CYTOLOGICAL STUDY OF ORANGE LEAF RUST, PUCCINIA TRITICINA PHYSIOLOGIC FORM 11, ON MALAKOFF WHEAT [1]

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INTRODUCTION

This paper reports the second of a series of cytological studies of orange leaf rust, *Puccinia triticina* Eriks. physiologic form 11, on wheat. The first (*4*) [3] reported a study of this strain of rust on a fully susceptible variety, Little Club; the present paper treats of a consistently resistant host, Malakoff; and the third is to deal with leaf-rust infections on Kanred wheat, which is variable in its reaction to this rust.

MATERIAL AND METHODS

Seed of Malakoff, C. I. 4898, was received from E. B. Mains April 22, 1924. The strain of leaf rust is the same as that used in the studies of this rust on Little Club. It was collected by W. W. Mackie at Eureka, Calif., in 1923, purified by making a single-spore culture, and carried on in the greenhouse. A specimen sent to E. B. Mains was identified as *Puccinia triticina* physiologic form 11.

Plants for cytological study were grown in the field. Seed was sown April 25, 1924, and the seedlings were inoculated May 4. The second lot was sown January 17, 1925, and the seedlings were inoculated February 26. For older material, seed was sown June 1, 1924, and the half-grown plants were inoculated January 14 and again February 19 of the following winter. The plants were kept covered with a bell jar for 48 hours after inoculation. Material was fixed at intervals from the time of inoculation until 18 days later.

The ordinary cytological technique was modified in a few details. Chrom-acetic-urea mixtures (usually 1 per cent chromic, 1 per cent acetic, and ½ per cent urea salts) were used as fixing fluids. Material fixed in the cold at 40° F. proved more satisfactory than material fixed at room temperature. In embedding, the pieces of infected leaf were placed in the interior of the paraffin block and not allowed to sink to its lower surface. This was particularly necessary in

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[3] Reference is made by numbers (italic) to "Literature cited," p. 713.

working with the tougher leaf tissue of grown plants, as it prevented tearing out during sectioning. Older leaf tissue is likely to break in sectioning, but by cutting exactly parallel to the veins, sections between one vein and the next can be obtained in good condition. The triple stain was used unless otherwise stated.

INVESTIGATIONS

Malakoff wheat is highly resistant to *Puccinia triticina* physiologic form 11 at all ages and under all the environmental conditions tested. Macroscopic evidence of infection is slow in appearing. A few small greenish flecks show about the sixth day in plants grown in May. These increase slowly in size and number, usually turning bright yellow. They remain less than a millimeter in diameter. In winter, the flecks form still more slowly. They do not appear for 8 or 10 days, and the great majority are less than one-fourth of a millimeter in size. Uredinia are rare and are of almost microscopic dimensions.

The three sets of rusted plants were grown at different seasons of the year and encountered different weather conditions. Cytological study shows points of difference.

The first set was planted in the field April 24, 1924, after an exceptionally dry season, and there was no rain during the growth of the seedlings. The plants were exposed to good light (71 per cent of the possible sunshine). They were watered just enough to prevent wilting.

Microscopic study showed that the spores germinated vigorously and that the germ tubes entered the stomata of the host promptly. Of 66 fungi which formed appressoria on the stomata, 60, or 91 per cent, had entered the host by the second day. Under these conditions, at least, the fungus enters Malakoff leaves freely.

The process of entry is not altered in any way by the fact that the host is resistant. It resembles closely the entry into a susceptible host (*4*).

Steps in this process are seen in Plate 1, A, B, and C. Two days after inoculation, as noted above, spores had germinated, the appressoria had formed, and the majority of the fungi had passed through the stomatal slit, formed the round substomatal vesicle, and pushed out the infecting hypha. A few were still at the stage represented in Plate 1, A. The drawing represents half of a guard cell, *b d*, and the fungus on it. The fungous plasm has passed between the guard cells and formed the substomatal vesicle, *c*, leaving the appressorium, *a*, empty and collapsed. Entry commonly takes place, as in this case, through one end of the stomatal slit. Six nuclei are seen in this section of the vesicle. The total number probably is eight.

EXPLANATORY LEGEND FOR PLATE 1

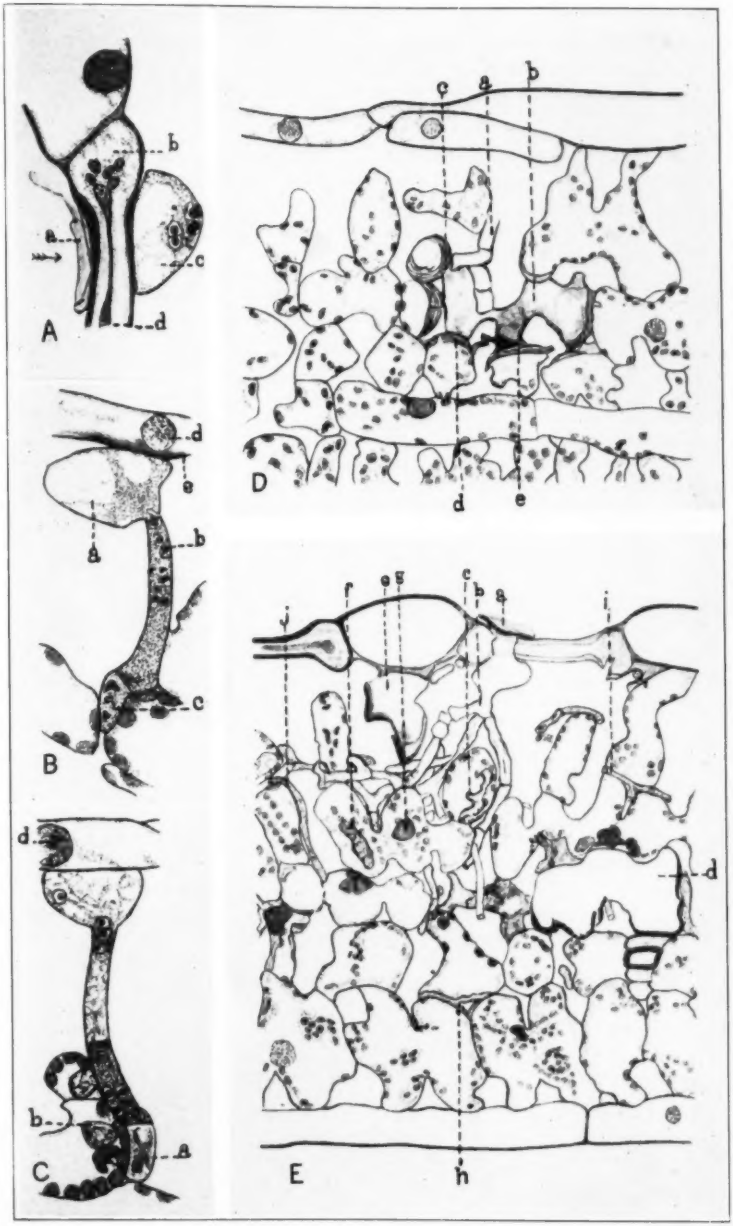
A.—Portion of guard cell, *b d*, bearing the empty appressorium, *a*, on its outer side and the substomatal vesicle, *c*, within. ×730

B.—Two-day infection. The substomatal vesicle, *a*, is in contact with the accessory cell of stoma, *e*. The infecting hypha, *b*, contains six nuclei. Haustorium mother cell, *c*, at its tip is wedged between two mesophyll cells. ×730

C.—A slightly later stage, also from a two-day infection. The haustorium mother cell is producing a young haustorium, *a*. The host nucleus, *b*, is near by. ×730

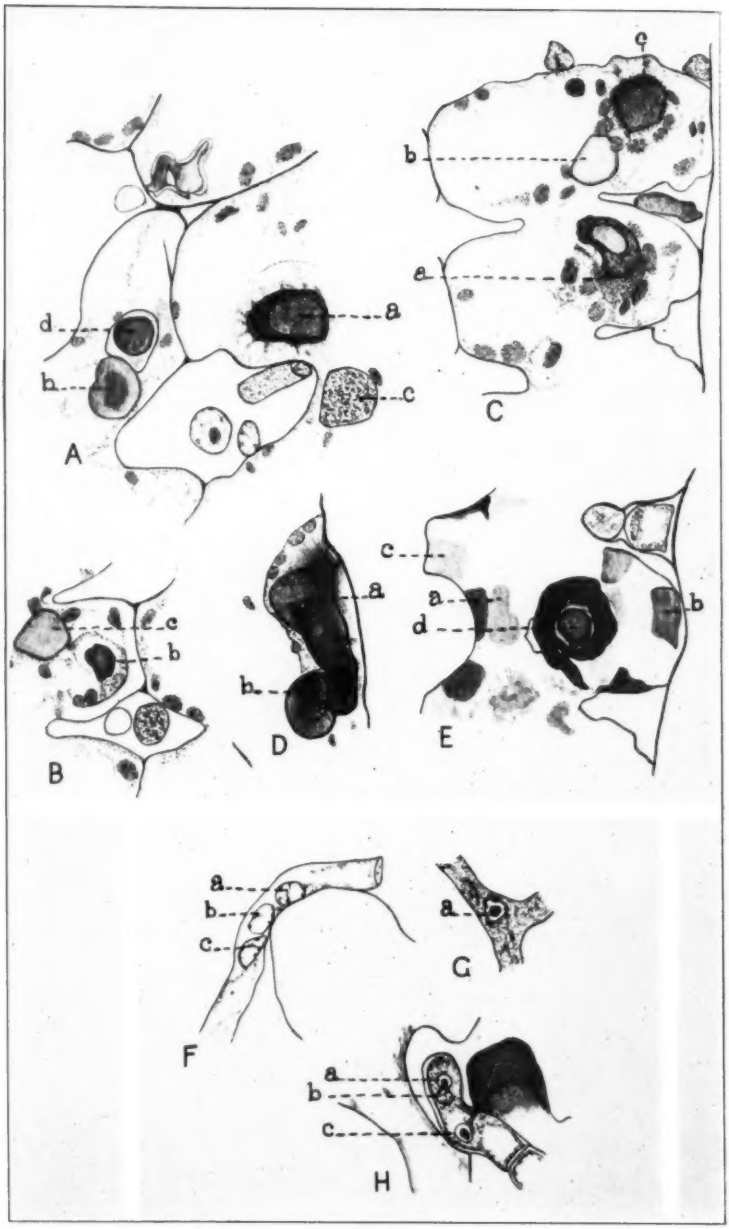
D.—Eight-day infection. The fungus has been dead several days. There is an empty swollen hypha at *a*, a dead host cell at *b*, and swollen walls *b*, *d*, and *e*, in contact with the dead cell. There is very little disturbance in the surrounding tissue. ×333

E.—Exceptionally large eight-day mycelium (over 300μ in diameter) with empty appressorium, *a*, and vesicle, *b*. The stoma is dead. The central mycelium is drained, but hyphae further out, *i* and *j*, contain cytoplasm. Haustoria at *c* and *f* have expanded partly. In cells *d* and *e* the contents have dissolved, leaving the cells empty. Host nuclei, *g*, die prematurely. Walls in contact with dead cells, as at *h*, may become swollen. ×333



Puccinia triticina physiologic form 11 on Malakoff wheat. Infections on seedlings grown in dry bright weather, in April and May, 1924

For explanatory legend see page 698



Puccinia triticina physiologic orm 11 on Malakoff wheat. Infections on seedlings grown in dry bright weather, in April and May, 1924

For explanatory legend see page 699

From this rounded, more or less hemispherical vesicle, the infecting hypha pushes out. No case has been noted in which more than one hypha forms from the vesicle. This hypha does not run over the end of the stoma and keep in contact with the inner surface of the epidermis, as is so commonly the case in stem rust, but instead it dips straight down into the large air space below the stoma.

In Plate 1, B, the vesicle, *a*, has pushed out the infecting hypha, *b*, which first encountered a mesophyll cell, then soon became wedged between two cells, and formed the short terminal haustorium mother cell, *c*. The hypha contains six or more nuclei, and the mother cell probably three. Plate 1, C, shows a slightly more advanced stage, and a young haustorium is forming at *a*. A portion of the host nucleus (divided in sectioning) is seen at *b* in contact with the haustorium. In both B and C the section was oblique and cut through an accessory cell of the stoma. It is noteworthy that in both of these accessory cells the host nucleus, *d*, lies at the point in the cell nearest to the fungus, as if attracted by it, and in the one at *e* the host-cell wall is injured (the injury is represented by shading). Even at this stage it is not uncommon to find a stainless area in guard-cell walls in contact with the fungus, which shows that these walls are altered chemically by fungous secretions.

In only one or two cases in the two-day material was the host cell in which a haustorium had formed killed. In four-day material, however, cells invaded by the fungus were killed and collapsed, and are seen in the preparations as irregular shrunken red-stained masses.

The formation of germ tube, appressorium, vesicle, and infecting hypha takes place without any food other than that stored in the spore. The first attempt of the fungus to establish food relations with its host is quite uniformly fatal to the host cell and to the haustorium within it. This wastes the slender resources of the young fungus, and occasionally it dies at this stage. In older material it is not uncommon to find minute dead infections consisting of an empty appressorium and substomatal vesicle at a dead stoma and one empty hypha leading to a single dead host cell.

Commonly, however, the fungus survives and forms successive haustoria in several cells. It may die at any time in this process, or continue a feeble existence for a longer period.

Plate 1, D, depicts the tissues in and around an eight-day infection. The fungus entered through the stoma in an adjoining section, and apparently has been dead for several days. One or two empty, swollen, transparent hyphae remain, as at *a*. Three or four host cells have been killed, one of which is shown at *b*. There is remarkably little disturbance in the surrounding tissues. Cell walls in actual contact with a dead cell, as at *c*, *d*, and *e*, may become greatly swollen, showing several lamellae, and the near-by contents of those cells may

EXPLANATORY LEGEND FOR PLATE 2

A to E.—Details from an 11-day infection, showing dead haustoria. Part of this mycelium is still livin× ×1130
A.—Haustoria are at *a* and *d*, a living nucleus is at *c*, and dead nucleus at *b*
B.—There is a small dead haustorium, *b*, accompanied by dead nucleus, *c*
C.—Haustoria at *a* and *c* surrounded by living cytoplasm of host cell. Dead nucleus at *b*
D.—Larger dead haustorium, *a*, accompanied by nucleus *b*
E.—Haustorium, *d*, in dead host cell. Disintegrated plastids at *a*, *b*, and *c*
F.—Hypha containing three nuclei, *a*, *b*, and *c*, with a coarse chromatin net and no nucleolus. ×1130
G.—Fungous nucleus, *a*, with both nucleolus and coarse chromatin net
H.—Hypha with two ordinary nuclei, *a* and *c*, and one nucleus, *b*, with heavier net and no nucleolus

be slightly damaged. Beyond this, the tissues look healthy. The entire affected area is microscopic in size and would not be visible to the unaided eye in living material.

If the fungus still is living eight days after inoculation, a much larger area may be involved. The diameter of the mycelium ranges from 100μ to 300μ. Even in the most vigorous mycelium, however, the growth is meager. Plate 1, E, is representative. The fungus entered at *a*, and the guard cells at the point of entry are dead. The single infecting hypha forked at *b*, the branches leading off into the tissues. Not even at the center of the infection are the intercellular spaces in the leaf filled with hyphae. All of the older mycelium is drained and empty, and some of the hyphae are wrinkled and misshapen. Only the marginal hyphae, as at *i* and *j*, are living, and they are not vigorous.

Haustoria are formed but often remain small and dense and die without expanding. A few expand partly, as at *c* and *f*. The average size of the five largest haustoria found was 16.4μ by 4.6μ, which is little over one-third the size of the largest in Little Club (45μ).

The host cells in which haustoria form are killed. The first cells invaded undergo a quick collapse and the cell contents become homogeneous and stain a dense red.

As has been noted in other rust studies (*2*, *3*), however, later attacks upon a resistant host, in any given infection, produce less violent results in the host cell than do the earlier attacks. But under these conditions, Malakoff and this form of leaf rust are so highly uncongenial that, even in an older infection, the life of an invaded cell is short. In the infection shown in Plate 1, E, all the cells containing haustoria were counted. About half of them were dead, and nearly all the rest showed marked signs of disorganization. The host nucleus underwent little or no expansion and soon died (pl. 1, E, *g*), and the plastids and cytoplasm diminished and disappeared, leaving the cell clear, colorless, and empty (pl. 1, E, *d* and *e*).

The effect of the fungus on host-cell walls at this stage is practically limited to the invaded cells. Occasionally the wall of an adjoining living cell is affected beyond the limits of its contact with the dead cell, as at Plate 1, E, *h*, but even this is not the rule. A close examination of these swollen walls shows that the outermost layers of wall are first affected. Often the inner layers, those nearest the protoplast, still take normal stain.

The damage done by the fungus at this time (eight days after inoculation) is virtually limited to the infected area and a few cells just beyond its edge. Plastids in newly invaded cells average 3.1μ by 1.8μ in size, those just beyond the marginal hyphae 3.9μ by 2.5μ, and the normal plastids 5.3μ by 2.7μ. No excess of starch has been observed either within or around the infection.

During the next few days there is a marked change in the appearance of both the fungus and its host. In 11-day infections a few of the mycelia are still living although they have increased but little in diameter. Within the infection both host and fungus show profound disturbances. The hyphae are impoverished in content and struggling for existence. Much of the mycelium is drained, and the empty hyphae are swollen and sometimes misshapen. Even in the growing tips one finds more or less vacuolated cytoplasm.

The growth is meager within the infected area, and the fungus is making but feeble attempts to spread to fresh tissues beyond. Stolons are virtually absent.

Nuclei of the fungus often are hard to see and in some cases have undergone a change of structure. The regular fungous nucleus (pl. 1, A, B, and C) consists of a nuclear membrane, a central spherical body, probably nucleolar in nature, and a fine, scarcely visible chromatin network. Some of the nuclei shown in Plate 2 still have this structure (pl. 2, H, *a*, *c*). In other nuclei, as in those shown in Plate 2, F, *a*, *b*, and *c*, and in Plate 2, H, *b*, the nucleolus has disappeared, and the chromatin network has become relatively coarse and heavy. Plate 2, G, at *a* apparently shows a transition stage. This change of nuclear structure was noted in some cases in eight-day infections but is much commoner in older mycelia.

When such a change of nuclear structure was first noted, it was considered possible that one or the other type of nuclear structure was the result of poor fixation. This may be so, but when one finds both types common in the same preparation, and even in the same cell (pl. 2, H), the correctness of such an interpretation becomes doubtful.

A similar alteration in the structure of the fungous nucleus takes place regularly in haustoria, even on susceptible hosts. The single haustorial nucleus contains a coarse chromatin net but no nucleolus. Perhaps this change is of a degenerative nature and occurs when the nucleus is no longer able to divide. Or it may be due to some adverse effect of the host, limited to the haustoria when the fungus is grown on susceptible hosts but tending to spread out into the mycelium when the host is more uncongenial.

In infections of this rust on Little Club (*4*), it was found that the rust nuclei are commonly in groups of three. Even on Malakoff, on which the fungus grows poorly and abnormally, groups of three nuclei are noted (pl. 2, F and H).

As in the younger mycelia, haustoria form, but nearly all soon undergo degenerative changes and die. The cytoplasm and nucleus of the haustorium disintegrate and form a nearly homogeneous substance that stains intensely with safranin. In shape the dead haustorium is usually shorter and thicker than the living haustorium. In some cases no structure can be discerned within the haustorium (pl. 2, D, *a*, and pl. 2, B, *b*). Other haustoria, as those shown in Plate 2, C, *a*, and Plate 2, A, *d*, seem to be composed of concentric layers with differing affinity for stain. Sometimes these haustoria are closely surrounded by cytoplasm and plastids of the host (pl. 2, C, *a* and *c*), while at other times they are fairly free (pl. 2, A, *d*). It is not certain whether the heavy coating of the haustorium in Plate 2, E, *d*, is derived from the haustorium, or the host, or both. The degree of this degeneration of haustoria varies with the vigor of the fungus.

The host nucleus may survive for a short period after the entrance of the fungus into the cell (pl. 2, A, *c*) but usually dies almost immediately (pl. 2, B, *c*). Whether living or dead, the host nucleus is to be found close to the haustorium. The plastids persist for a time, but ultimately disintegrate into red-staining smears (pl. 2, E, *a*, *b*, *c*). Many of these host cells collapse into irregular red-staining masses within which the outline of the haustorium may still be discerned.

The most striking changes 11 days after inoculation, however, are in the tissues beyond the fungus. Plate 3, A, represents a narrow

strip through the leaf from one epidermis to the other, at a distance of 150μ beyond the edge of the mycelium. The cells are living and are in fair condition, but the walls are irregularly swollen. Isolated spots have thickened into little wartlike prominences (*b* and *c*) which stain almost black in the triple stain. Occasional wider areas of wall, as at *a*, *d*, or *e*, fill in a small intercellular space or form a wedge-shaped mass between two lobes of a cell. These swellings occur only in the proximity of the infections in a conspicuous zone, which varies in width from a few microns to 300μ.

The disturbance obviously is due directly or indirectly to the fungus. It is noteworthy, however, that, although each infection is surrounded by a zone with swollen walls, the tissue in the infection itself has little or none of this swelling. (Compare pl. 3, A, with pl. 2, A to H.)

Measurements were made of nuclei and plastids in and around one of these infections. The nuclei of the outer zone show no abnormal expansion in size, the average being 9.5μ by 7.3μ, as compared with 8.9μ by 7.9μ in the healthy tissues beyond. There is remarkably little decrease in cytoplasm and plastids. Plastids in living invaded cells averaged 2.7μ by 1.8μ; just beyond the marginal hyphae they averaged 2.8μ by 1.8μ; 100μ beyond the fungus they averaged 4.2μ by 1.9μ, and the size of plastids in healthy tissue in this leaf was 4.3μ by 2.1μ. Thus it would appear that the partial dissolution of host-cell walls extends much farther beyond the fungus than does the impoverishment of the cell contents, for the cell contents look normal 100μ beyond the fungus, whereas swollen walls may occur for a distance of 300μ. No excess starch was found in plastids of tissues affected by the fungus.

A week later (18 days after inoculation) the effects had become more marked. Plate 3, B, represents a strip through the leaf at the center of the infection, and Plate 3, C, is drawn from the zone of tissue surrounding the same infection, at some distance beyond the outermost hyphae. The leaf tissues of both are dead. At the center, a few remnants of hyphae are still visible. Some of them, as those in Plate 3, B, *a*, still show small amounts of cytoplasm. The mesophyll cells have collapsed and are misshapen, and even the epidermis has flattened out.

In the outer zone (pl. 3, C), which sometimes is 300μ or even more in width, the cell walls usually have not collapsed, but the contents are shrunken and dead, and the walls are thickly covered with coarse black-stained "warts," many times the number and size of those found in the 11-day material. This zone with swollen walls is conspicuous even under the low-power lens of the microscope.

As has been noted already, the swellings on the walls extend outward into intercellular spaces or form between adjoining cells at contact surfaces. This suggests that it is the first-formed layers of wall (the middle lamella and to some extent the secondary lamellae

EXPLANATORY LEGEND FOR PLATE 3

A.—A narrow strip through the leaf 150μ beyond an 11-day infection. The tissues are living, but there are localized wartlike swellings on the walls (*b*, *c*) and larger swellings at *a*, *d*, and *e*. ×333

B.—Drawing of leaf section at center of 18-day infection. The tissues are dead. There are remnants of hyphae at *a*. ×333

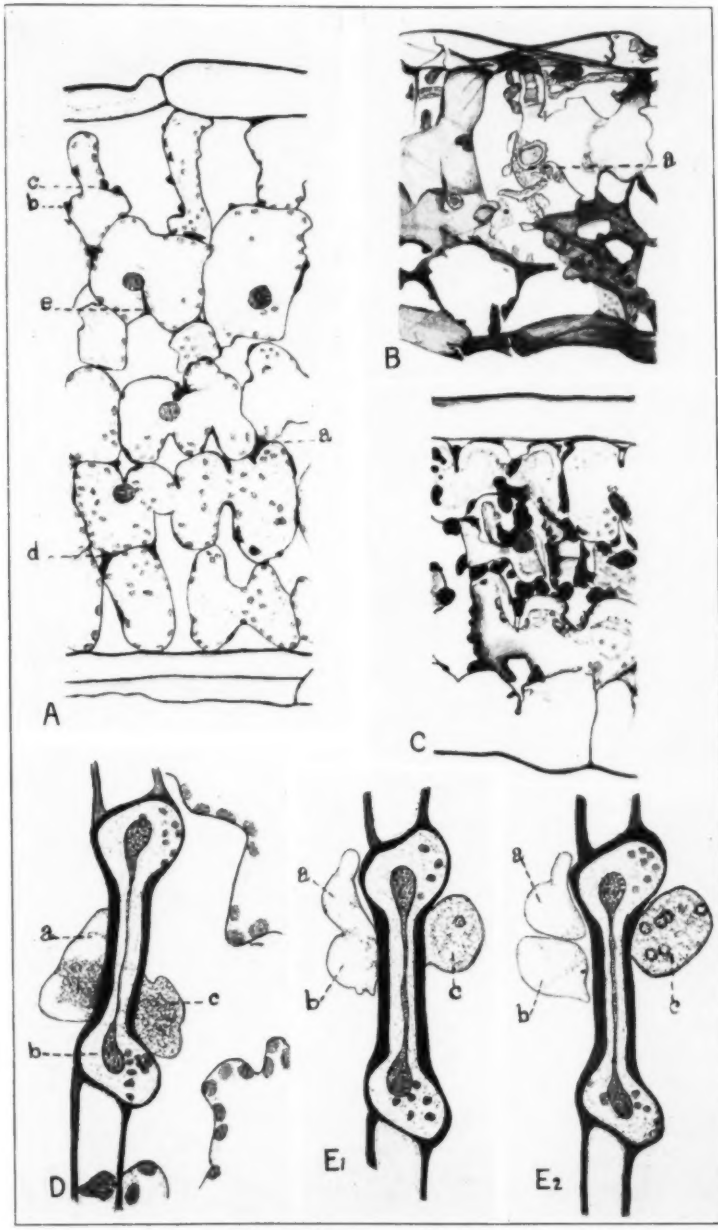
C.—Portion of the area surrounding the infection drawn in B. Extensive breakdown of pectic materials of mesophyll cell walls. The cells are dead. ×333

D.—Stoma, *b*, with appressorium at *a* and a vesicle forming at *c*. ×730

E_1 and E 2.—Two views of the same stoma

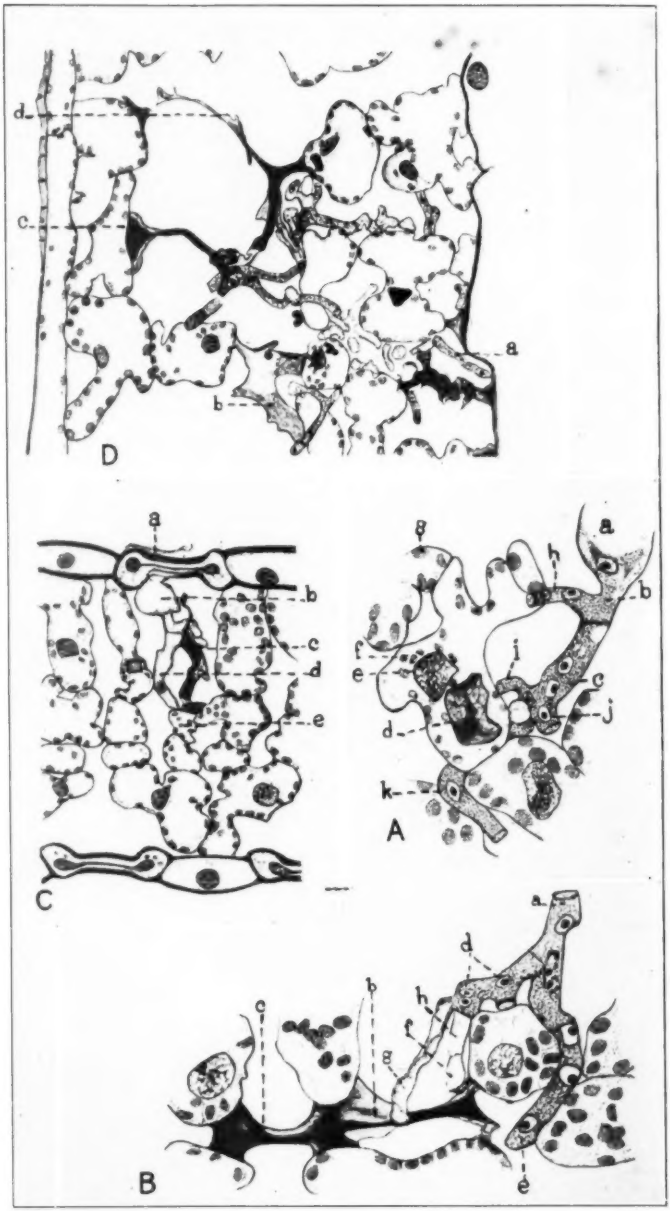
E_1.—The center of the appressorial mass, *a b*, and a side section of the vesicle, *c*

E_2.—A side section of the appressorial mass, seen as separate masses, *a* and *b*, and the center of the vesicle, *c*. ×730



Puccinia triticina physiologic form 11 on Malakoff wheat. A, B, and C, infections on seedlings grown in bright dry weather, in April and May, 1924; D and E, infections on older plants grown in dark wet weather, in February, 1925

For explanatory legend see page 702



Puccinia triticina physiologic form 11 on Malakoff wheat. Infections on older plants grown in dark wet winter weather, in January to March, 1925

For explanatory legend see page 703

next to it) that are being decomposed. If so, these swellings should contain pectic compounds (*12, 15, 1*) and take the stains for pectose and its decomposition products. The inner, later layers of wall appear to be untouched.

When sections of this material were stained with methylene blue, washed, and mounted in water, the swellings varied from violet to deep purple. This is a typical pectic reaction. Sections were stained lightly with ruthenium red, dehydrated, and mounted in balsam. If the staining is done properly, the cell contents are colorless and the wall swellings are a clear red. This also indicates the presence of pectic materials. Also, it explains the bulkiness of these decomposing walls, for the ability of pectin to swell and gelatinize is well known. With both stains, the most completely decomposed wall substances, i. e., the swellings formed earliest, took little or no color.

An attempt was made to duplicate the condition of these walls artificially. Sections of wheat leaf were subjected to partial maceration in a mixture of absolute alcohol and hydrochloric acid and then washed and stained. Thickened middle lamellae were noted, with larger swellings at the edges, but no typical "warts" were found. Mangin, however, describes an experiment in which were produced results apparently resembling the work of the fungus in Malakoff infections. He says (*12, p. 297*):

> La surface externe de la membrane qui limite les méats présente souvent des sculptures formées par des pointes ou des boutons de pectates insolubles, dispersés sans ordre à la surface ou, le plus souvent, fixés sur les bords des cadres d'union. * * * Enfin, dans un certain nombre de cas, les méats sont partiellement ou complètement remplis d'une sorte de gelée due à la transformation de l'acide pectique normalement insoluble, en une masse capable de se gonfler et de se dissoudre dans l'eau. * * *

Ordinarily, the rust dies without attaining even the beginnings of reproductive activity. Only rarely does one find even a minute uredinium. Host tissues die and fungous growth stops after the formation of a few spores. The epidermis of the host may not even be ruptured above the spores.

Infections on older hosts were studied during the following winter. The seed was sown June 1, 1924, in the field, and the plants grew slowly through the summer and fall, forming a heavy rosette. By January 14, 1925, when the first inoculations were made, the plants were pushing up culms and stood 2 feet high. Later leaves on these plants were inoculated a month later (February 19). Material from both lots was fixed at stated intervals after inoculation.

The fungus developed slowly under winter conditions. A few minute, bright-yellow flecks had appeared by the tenth day. These increased slowly in number and size, only a few exceeding a fourth of a millimeter in diameter. No hint of spore production was noted.

For unknown reasons, perhaps associated with winter conditions, the hyphae of a considerable percentage of the appressoria in this

EXPLANATORY LEGEND FOR PLATE 4

A.—Detail of five-day infection showing first attack. The substomatal vesicle is at *a*, the infecting hypha, at *b c*, the first haustorium (dead) at *d*, attended by the host nucleus, *e*. Plastids of the host cell, *f*, are minute. The infecting hypha has branched at *h, i, j, k*. ×730

B.—Slightly later stage. Infecting hypha, *a, f*. The host cell, *b c*, is dead. The haustorium mother cell *f*, and hypha, *h*, are empty. The branch, *g*, is enfeebled. More vigorous branch at *e*. ×730

C.—Portion of leaf section six days after inoculation showing collapsed appressorium, *a*, vesicle, *b*, hypha, *d*, dead host cell, *c*. The fungus is nearly dead. ×333

D.—Seven-day infection that is gaining ground. There are dead host cells at *b, c*, and *d*, and fairly vigorous hypha at *a*. Surrounding tissues show little disturbance. ×333

material failed to enter the stomata, but when entry took place it resembled closely what has already been described. In Plate 3, D, the appressorium, *a*, has formed on the guard cell, *b*, and its contents are moving through the stomatal slit to form the substomatal vesicle, *c*.

Plate 3, E_1 and E_2, recalls at once the cases of fusion of appressoria seen in material of this rust growing on Little Club (*4*). Successive sections of the same stoma are shown. At the center of the appressorial mass (pl. 3, E_1), it appears as a two-lobed figure, *a* and *b*, somewhat constricted at the center. At either side of the center, as in Plate 3, E_2, it appears as two separate masses, *a* and *b*. A single substomatal vesicle, *c*, is forming inside. Appearances suggest strongly that two appressoria fused and are entering as one.

From the rounded vesicle just inside the stoma a single multinucleate infecting hypha pushes out and soon forms contact with mesophyll tissue. As before, the first cell attacked is killed. Plate 4, A, drawn from a five-day infection, shows a step in this process. From the partly evacuated vesicle, *a*, proceeds the hypha, *b c*. It has formed a haustorium, *d*, already dead and partly disorganized. The host cell in which it formed is living but shows marked disturbance. The nucleus and part of the cytoplasm have moved to the haustorium. The plastids, *f*, are reduced in size almost to extinction but, oddly enough, contain a minute amount of starch, in fact more than the healthy plastids of surrounding cells, *g*. Excess starch is fairly common under some conditions in infections on other hosts, but it is rarely observed on Malakoff. The fungus in this case is still alive, and the infecting hypha has produced three or four branches (pl. 4, A, *h*, *i*, *j*, *k*).

In Plate 4, B, also from a five-day infection, is shown a more advanced stage of development. The hypha grew from *a* to *f* and formed a haustorium in the cell, *b c*. This host cell is dead and has collapsed into a stringy mass. Moreover, the haustorium mother cell at *f* has died and nearly disappeared. Apparently the fungous cytoplasm below this cell retreated and formed a septum near *h*. A branch, *g*, has formed near this septum but is in feeble condition. Farther back, a more vigorous branch, *e*, is growing out.

It is noteworthy that often, even under conditions so unfavorable to the fungus that it dies, there is a marked tendency toward the trinucleate condition. Plate 4, A and B, and Plate 5, A, show a number of fungous cells with three nuclei each.

The growth of the fungus is slow and very variable. In Plate 4, C, is shown part of a longitudinal section of the leaf containing a six-day infection. A withered appressorium at *a*, an empty vesicle at *b*, one or two empty hyphae, *d*, and a dead and dried host cell at *c*, and that is all. One other hypha is found in the next section. The fungus is practically dead. Except for a slight reduction in plastid size as at *e*, the adjoining cells are normal.

As was the case when the seedlings were infected, a considerable percentage of the fungi died during the first week. In the later fixations were found minute dead infections consisting, like the one in Plate 4, C, of an empty substomatal vesicle, one or two empty and partly disintegrated hyphae, and, associated with them, one or two collapsed host cells. Sometimes only traces of the former hyphae remained.

If, however, the fungus remains alive through the first week, it stands a fair chance of living much longer, for a process of adjustment takes place here similar to that described above in connection with Malakoff seedlings and other rusts (*2*, *3*). Invaded host cells live a little longer, and haustoria expand partly and extract some food for further growth before the host cell dies.

The infection shown in Plate 4, D, is 7 days old and is making progress. The mycelium extends through several sections. It is a sparse growth, but there are several living hyphae, and the fungus is slowly gaining strength. The host cells die soon after being invaded (*b*, *c*, *d*) but not until the fungus has extracted enough food to enable it to grow to the next cell. No gelatinization of host-cell walls either within or beyond the infection was noted at this stage, and the impoverishment of tissues beyond the infection was negligible.

A week later, in 14-day infections, the fungus had not increased greatly in diameter. A few mycelia were measured and were found to range from 120μ to 300μ in diameter. In the one shown in Plate 5, B, the fungus was still alive, but some hyphae were dead (*h*), and others were empty or thin in content. Many of the haustoria fail to expand but undergo the type of degeneration seen so commonly in the seedling material. An increasing number, however, expand partly and function for a while. The average of the largest is 34μ, whereas the maximum on a congenial host is close to 50μ.

The damage done to the host is localized within the minute area occupied by the fungus. In this area, cells are dead or dying. The cells that were first attacked have collapsed (*e* and *j*). Within the smeared-looking, red-stained contents of these cells one sometimes sees remnants of nucleus and haustorium (*j*). Not all of the cells entered later by the fungus collapse in this fashion. Instead, the nucleus dies, the plastids dissolve (pl. 5, B, *k*, *i*), and the cell may become clear and empty (*f*). This is a slower and apparently less extreme reaction to the fungus and is found only in older infections. Occasionally a wall in contact with a dead cell (*a* and *d*) will swell up, showing the lamellae of which it is composed.

The impoverishment of tissues surrounding the infection is slight. Two or three cells beyond the fungus (pl. 5, B, at top of drawing) the tissues are nearly normal. The plastids are slightly decreased in size and the nuclei are somewhat expanded, measuring 10μ by 8.8μ as compared with the normal size of 9μ by 7.6μ.

The cell walls in this outer zone are virtually unchanged, although here and there one finds minute swellings (*c*, *l*) of the sort so abundant near the seedling infections.

The oldest infections studied on these plants were 19 days of age. The rust had been growing faster and at this time the infections ranged from 150μ to 750μ in diameter. This increase took place by the slow spreading of feeding hyphae, no runners having been noted.

At the center of the larger 19-day infections both host and fungus are dead. That shown in Plate 5, C, is typical. The host cells have collapsed and their broad lobes have shriveled into strands (*b*, *c*). Within these cells occasional remnants of small spherical haustoria, *a*, are still visible. Hyphae have increased in number. They are dead and empty but still persist. There seems to be little disappearance of mycelium here.

Out toward the margin of this same infection, intermingled with collapsed cells, are a few which have retained their original shape (pl. 5, D). They are deficient in contents but not plasmolyzed. The nucleus usually is dead (pl. 5, D, *b*, *c*), and the plastids are minute. In appearance they resemble fairly closely the corresponding tissues in an old infection on a susceptible host. The development of the haustorium, too, had run a nearly normal course; it had expanded partly (it was 25μ long), had functioned without causing collapse of the host cell, and had undergone normal drainage, leaving it empty and clear.

In the beginning, haustoria remained as minute dense bodies which died without expansion. Some gradual change in the relation of host and parasite made possible greater expansion and more normal functioning of haustoria and, correlated with it, an increased rate of growth of the hyphae. At its best, however, the fungus makes but a feeble advance, and even in old infections, some host cells die soon after being entered. In Plate 5, E, at the very edge of the infection, are two collapsed cells, *a* and *b*.

Swellings on host-cell walls are still few and occur more commonly just beyond the infection than in it. The cells in Plate 5, D, have unmodified walls, although they are surrounded and invaded by the fungus, whereas the uninvaded cells in Plate 5, E, *c*, *d*, and *e*, bear little hemispherical swellings. Warts do occur on walls of attacked cells (pl. 5, E, *a* and *b*) but may have formed before the fungus reached them. Certainly the great majority of warts are found on cells not in direct touch with the fungus. The disintegration of host-cell walls is a minor factor in this material.

On the whole, the development of the organism is sharply restricted. In many instances the organisms die during the first week. The few that survive gain slowly. The host tissues at the center of the mycelium die, but in the further spread of the fungus some sort of tolerance is developed that allows haustoria to expand more and take more food and the hyphae to grow faster. The average diameter of the 19-day mycelia is 429μ, nearly twice that of the seedling infections of the same age. Although the vegetative growth of the fungus is greater than that in the seedling infections, no trace of reproductive activity has been found here.

On comparing the infections on seedlings grown in the spring with those on older plants in the winter, differences are noted. Old infections on seedlings are small and are surrounded by a broad zone of tissue whose walls undergo partial disintegration and whose contents die. Infections of the same age on grown plants are larger. They have but a small trace of this disintegration of walls, and the tissues around the infection are living. It is not clear whether these differences are due to the difference in the age of the hosts or to differences in weather conditions.

EXPLANATORY LEGEND FOR PLATE 5

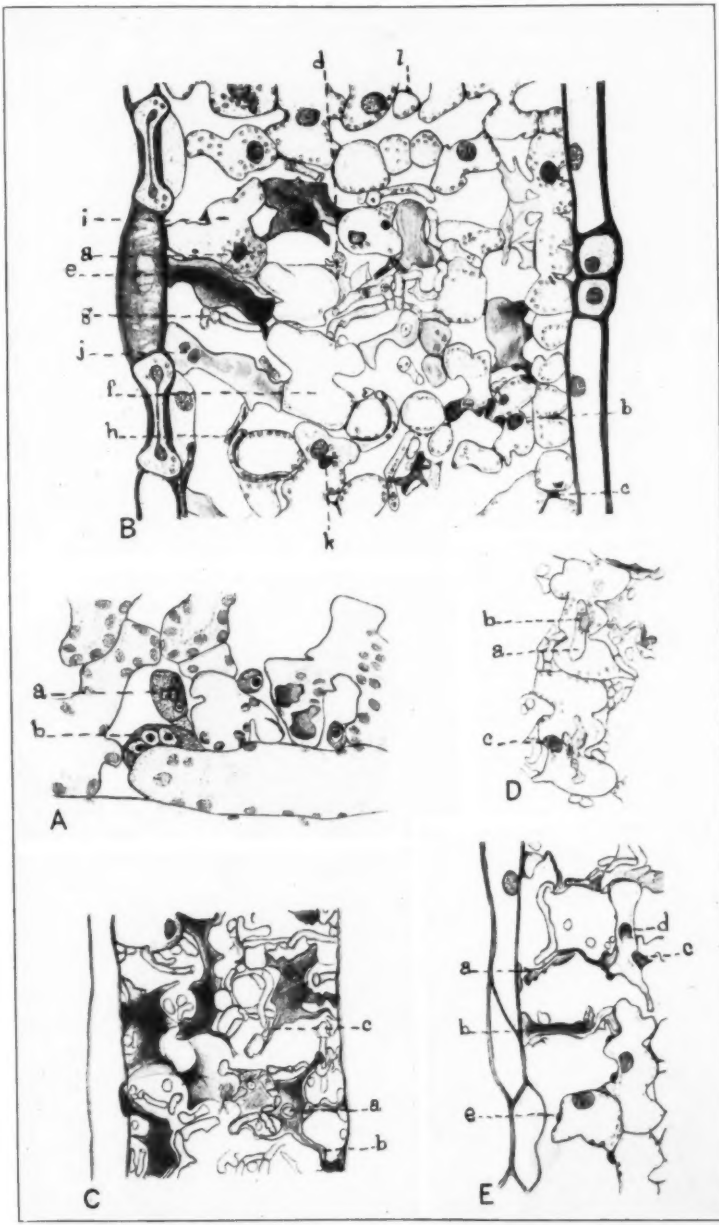
A.—Detail of five-day infection, showing haustorium mother cell at *a* and hypha with three nuclei at *b*. ×730

B.—Section through 14-day infection, showing collapsed cell at *e*; one with remnant of haustorium at *j*; cells at *i* and *f* not collapsed but with contents dissolving; swollen walls in contact with dead cells at *a* and *d*; and small swellings of walls at *c* and *l*. Some hyphae are living, some, at *h*, are dead, and others, at *g*, are drained. ×333

C.—Section through center of 19-day infection. Host and fungus, dead. Haustorium at *a*, dead. Lobes of cells at *b* and *c* narrowed into strands. ×333

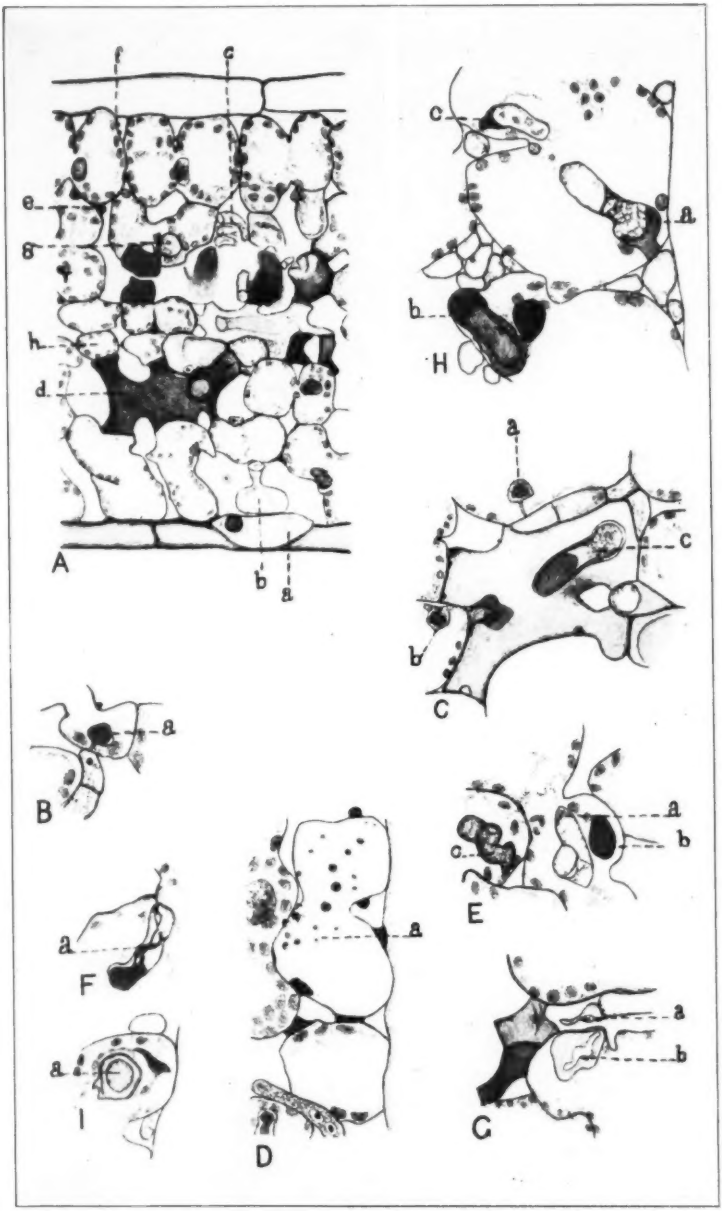
D.—Detail from same infection as C but out toward margin. Cells not collapsed nor walls swollen. Drained haustorium at *a*. Dead nuclei at *b* and *c*. ×333

E.—Detail of same mycelium at the margin and beyond it; *a* and *b* are collapsed cells; *c*, *d*, and *e* are swollen "warts" of pectic material. ×333



Puccinia triticina physiologic form 11 on Malakoff wheat. Infections on older plants inoculated during dark wet weather, in January and February, 1925

For explanatory legend see page 706



Puccinia triticina physiologic form 11 on Malakoff wheat. Infections on seedlings inoculated during dark wet weather, in January, 1925

For explanatory legend see page 707

Infections on a third set of plants served as a check. The seed was sown beside the older plants January 17, 1925. The seedlings were inoculated on February 26, and encountered the same dark wet weather as the infections on the older plants.

Here, as before, macroscopic evidence of infection was slow in appearing. Even on the fourteenth day the flecks were few, minute, and vague. On the eighteenth day they had turned bright yellow, but even the largest was less than a millimeter in diameter.

Microscopic study shows that the fungus experiences the same severe struggle for existence during its early life that was seen in the other material. Even on the ninth day the fungus was not well established in the host. Plate 6, A, represents the central section through a nine-day infection. The fungus entered through the stoma of which *a* is an accessory cell. The empty substomatal vesicle, *b*, and a few empty hyphae, such as those at *c*, make up the sparse growth of the fungus at the center. Marginal hyphae in adjoining sections look more vigorous, but there are only a few of them. Host cells collapse very soon after they are entered (pl. 6, A, *d*). Young haustoria are drawn in Plate 6, B, at *a*, and Plate 6, C, at *a* and *b*. Haustoria may expand partly (pl. 6, C, *c*) but, as in this case, are soon checked by the dissolution of the host cell.

The disturbance caused by the fungus extends but little beyond the infection itself. Near-by host nuclei are enlarged somewhat (pl. 6, A, *g*), plastids may be reduced (pl. 6, A, *h*), and an occasional cell wall bears one or two wartlike swellings (pl. 6, A, *e* and *f*). An exceptional cell at the edge of the mycelium (pl. 6, D, *a*) bears numerous little swellings. The cell is living, but the contents have been omitted for clearness. In general, wall disturbances were slight and extended only two or three cells beyond the fungus.

On the fourteenth day the fungus was in somewhat better condition. The largest living haustoria averaged 15μ in length, but they often were twisted and irregular (pl. 6, E, *a* and *c*) and soon showed signs of degeneration (pl. 6, F, *a*). The final condition is shown in Plate 6, G, *b*, where the haustorium has changed into a short, irregular lump, taking little or no stain, and within which a vague concentric stratification can be discerned. The haustorium mother cell outside (pl. 6, G, *a*) is crumpled and shrunken. The host cell containing this haustorium has not collapsed.

On the fourteenth day a narrow zone around the infection showed slight impoverishment. There was very little breakdown of wall materials.

In the 18-day material about 20 living infections were studied. Mycelia had attained greater size in this than in any other Malakoff material studied. The largest mycelium was nearly a millimeter in

EXPLANATORY LEGEND FOR PLATE 6

A.—Center of a nine-day infection. The accessory cell of the stoma of entry is at *a*, the substomatal vesicle, at *b*, a few drained hyphae, at *c*, a dead host cell at *d*, swellings on the walls, at *e* and *f*, and expanded host nucleus, at *g*. ×333

B.—Detail of nine-day infection, showing young haustorium at *a*. ×730

C.—Half-grown haustorium, *c*, checked in its development by death of host cell. Young haustoria at *a* and *b*. ×730

D.—Cell at edge of nine-day infection, covered with numerous minute wartlike swellings of outer pectic wall layers. ×730

E.—Haustoria, *a* and *c*, somewhat twisted and contorted. Dead nucleus at *b*. ×730

F.—Haustorium in early stage of degeneration. ×730

G.—Detail from 14-day mycelium. Crumpled haustorium mother cell at *a*. Degenerated haustorium, showing concentric stratification at *b*. ×730

H.—An 18-day infection, showing stages of degeneration of haustoria at *a*, *b*, and *c*. ×730

I.—Dead haustorium from 18-day infection. ×730

diameter and the average was about 660μ. Much of this increase had come in the last few days, and on the whole there was an appearance of greater congeniality than there had been earlier.

The fungus had made relatively rapid growth, but with few exceptions the mycelium was not dense. The hyphae had normal diameters, but the cytoplasm was vacuolated. In general, only the growing tips of the hyphae had dense contents. Probably 95 per cent of the hyphae were drained and empty. Haustoria formed and expanded to greater size than in the younger infections. The average of the 10 largest found in one infection was 24.1μ. This, to be sure, is but little over half the size attained by haustoria in a susceptible host, but it is much greater than was found in younger infections in this material. Nor does this mean merely that haustoria take all this time to grow up. The earlier-formed haustoria were dead by this time. These haustoria were closer to the margin of the mycelium and were of later growth. Some of these haustoria (pl. 7, B, *a*) had a normal appearance and underwent normal drainage. The host cell was still vigorous, and its contents were normally disposed.

But here, too, degeneration slowly sets in. The neck and the basal end of the haustorium break down into matter that stains dull blue (pl. 6, H, *a*). Gradually more and more of the haustorium becomes involved, the change sometimes progressing faster along the surface than in the interior. Finally the whole haustorium becomes changed to a blue-staining mass consisting of an outer layer and an apparently homogeneous interior (pl. 6, H, *b*, and I, *a*, and pl. 7, C). It is still uncertain whether this outer layer was derived from host, or fungus, or both.

In tissues back nearer the center of the infection, that have been exposed longer to the fungus and are nearly spent, the fungus may still attempt to form haustoria. The hyphae contain little cytoplasm, but it is enough to form a haustorium mother cell. In Plate 7, D, there is an old dead haustorium at *a* and the beginning of a new one at *b*. The contents of the haustorium mother cell at *c* have only begun to enter the host cell, but already the mother cell has been altered chemically (it stains bright blue) by some substance that has diffused outward from the host cell. These blue-staining mother cells are of frequent occurrence in this area.

Even here where the fungus attains its greatest vegetative activity, the majority of the mycelia show no hint of reproductive activity. Occasionally there is a slight massing of hyphae below the epidermis. In only one infection (pl. 7, A) was a small uredinium (450μ in diameter) formed. Even in that instance the first spores died without maturing, but later spores looked more normal.

As may be seen from the drawing (pl. 7, A), nuclear phenomena in connection with reproduction are fairly regular. Spores, stalk cells, and some of the cells beneath are usually binucleate. There are exceptions. Plate 7, E, for instance, shows a stalk cell with three

EXPLANATORY LEGEND FOR PLATE 7

A.—Section through a small uredinium. Spores and stalk cells binucleate. Majority of host cells not collapsed. ×333

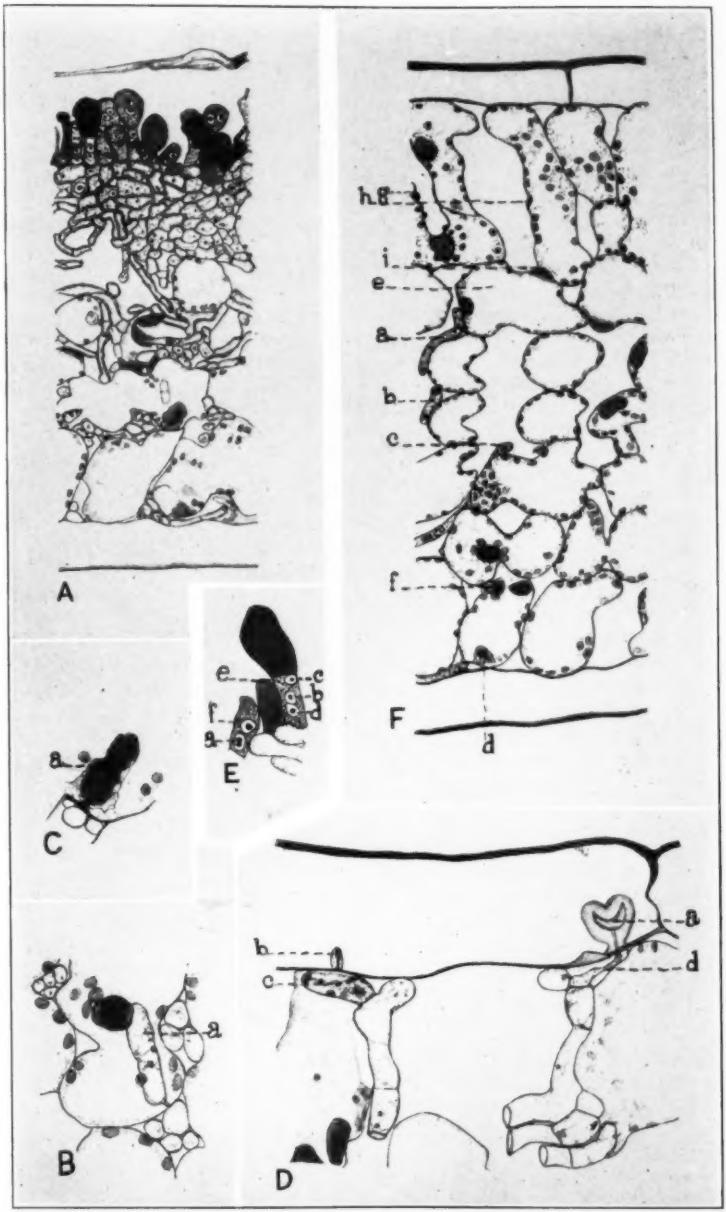
B.—Detail of 18-day mycelium, showing haustorium of normal appearance. ×730

C.—Dead haustorium with sheath. ×730

D.—Detail near center of 18-day infection. Old dead haustorium at *a* from collapsed mother cell at *d*. Beginning of a new haustorium at *b*. Blue-stained haustorium mother cell at *c*. ×730

E.—Detail from uredinium, showing a stalk cell with three nuclei, *b*, *c*, *d*, and a doubtful fourth nucleus at *e*, and a spore mother cell with one nucleus, *f*, and a second, *a*, containing two nucleoles. ×730

F.—Marginal region of 18-day infection. The outermost hyphae are at *a*, *b*, *c*, small haustoria at *d* and *f*, and slight swellings of walls at *g*, *h*, and *i*. The host tissues are living. ×333



Puccinia triticina physiologic form 11 on Malakoff wheat. Infections on seedlings inoculated during dark wet weather, in January, 1925

For explanatory legend see page 708

good nuclei, *b*, *c*, and *d*, and a doubtful fourth nucleus at *e*. In the same figure is a spore mother cell with one ordinary nucleus (*f*) and a second (*a*) which looks double and contains two distinct nucleoli.

Out in the mycelium there are more frequent irregularities in nuclear content. There are more binucleate cells in Malakoff than in Little Club. Cells of the hyphae contain anywhere from two to eight nuclei, and the haustorium mother cells may have two, three, or four. This breakdown in the regularity in the number of nuclei probably is associated with the adverse conditions under which the fungus lives.

In the later-invaded areas, the host, too, shows evidence of somewhat greater congeniality. The damage done during the early period still persists, of course, at the center of the infection, but as the fungus spreads outward to fresh tissues the host cells show progressively increasing tolerance. In the earlier reaction as the cell collapses the cell contents break down rapidly into a homogeneous substance that stains intensely with safranin. That occurs but rarely later on. In all the marginal regions of the fungus, host cells maintain their original size and form. The majority of invaded cells are still living, although the plastids tend slowly to decrease in size, the nuclei die prematurely, and in the end the cells become clear and empty.

Apparently the shift toward susceptibility is not great enough to bring into play the heightened cell activities seen on susceptible hosts. The normal nuclei of healthy tissue average 9.6μ in diameter, the living nuclei in cells bearing haustoria average 8.6μ, and the dead nuclei average 6.1μ. So far as can be judged by this, the nuclei have not expanded but have undergone slow contraction and death. The plastids decrease in size, and at no time has excess starch been seen in them. These cells, however, do show higher osmotic pressure than does healthy tissue.

The area immediately surrounding the fungus (pl. 7, F) shows almost no disturbance. The outermost hyphae are at *a*, *b*, and *c*, and there is a small haustorium at *d*. The cell at *e* has diminished protoplasm, and the one at *f* a dead nucleus. At *g*, *h*, and *i* are localized swellings of the pectic layers of the walls. Apart from that, the tissues appear healthy. This is in striking contrast to the appearance of the outer zone in the 18-day infections on the first set of seedlings studied (pl. 3, C), the tissues of which were dead and the walls heavily coated with large wartlike swellings.

In order to bring together the facts bearing on this point, the data have been tabulated (Table 1). The first set of seedlings had strong light, no rain, the minimum of watering, a mean humidity of 72, and an average temperature range of 50° to 65° F. Under these conditions the mycelium spread little, the average extent at 18 days being 235μ. A few infections produced minute uredinia, but each infection was surrounded by a broad zone of tissue in which the walls showed pronounced disintegration, and the contents were dead by the 18th day. The second set of seedlings and the older plants during the period following inoculation encountered short days and dark weather, over 11 inches of rain, a mean humidity of 79, and an average temperature range of 49° to 60° F. On the older plants the infections had nearly twice the diameter (429μ) of those of the first seedlings but bore no spores. The diameter of the infections on the second set of seedlings (661μ) was nearly three times that on the

first lot. In both, the effect upon the walls in the area surrounding the infection was slight, and the tissues were still living 18 days after inoculation. It would appear, then, that weather conditions have a greater effect than the age of the host in producing the differences noted.

TABLE 1.—*Dates of operations, summary of the weather conditions, and their effects on three lots of infections by Puccinia triticina physiologic form 11 on Malakoff wheat*

DATES OF OPERATIONS

Item	First seedlings	Second seedlings	Older plants
Date of planting	Apr. 23, 1924	Jan. 17,1925	June 1, 1924.
Date of inoculation	May 4, 1924	Feb. 26, 1925	Jan. 14 and Feb. 19, 1925.
Date of last fixation	May 22, 1924	Mar. 16, 1925	Mar. 16, 1925.

SUMMARY OF WEATHER CONDITIONS

Rainfall	0 inches during the experiment. The plants were watered only enough to prevent wilting. (Rainfall below normal in preceding winter.)	From Jan. 14 to Mar. 16 11.57 inches (heaviest rainfall between Feb. 3 and Feb. 23).	From Jan. 14 to Mar. 16 11.57 inches.
Temperature: [1]			
Average of daily maxima	65° F	60° F	60° F.
Average of daily minima	50° F	49° F	49° F.
Percentage of possible sunshine [1]	April, 73 per cent May, 69 per cent	January, 73 per cent February, 55 per cent March, 79 per cent	January, 73 per cent. February, 55 per cent. March, 79 per cent.
Mean humidity [1]	April, 70 May, 74	January, 82 February, 80 March, 74	January, 82. February, 80. March, 74.

EFFECT ON INFECTIONS

Average diameter of living 18 or 19 day mycelia	235μ	661μ	429μ.
Disintegration of pectic wall materials in zone surrounding infection	Pronounced	Slight	Slight.
Spore formation	Very little	Very little	None.

[1] San Francisco reports.

DISCUSSION

Malakoff wheat is highly resistant to *Puccinia triticina* physiologic form 11 at all ages and under all environmental conditions tested by the writer. Under the microscope, however, there are differences in the appearance of the infections developed under different weather conditions. Chief among these is the difference in the pectose reaction, for in one set of infections the swelling and gelatinization of pectic wall materials is pronounced; in the other two, it is relatively slight.

At least nine-tenths of the decomposition of host-cell walls occurs in cells beyond the mycelium. The fact that a cell attacked by the fungus bears "warts" does not prove that these swellings did not form before the fungus reached it. It seems safe to infer, however, that this breakdown of pectic compounds is due directly or indirectly to the fungus, as it occurs only in the neighborhood of infections.

It is conceivable that acids formed during the breakdown and death of host cells at the center of the infection might be capable of producing such an effect, but, on such a supposition, it is not clear why they do not produce greater effects on the cells within the infection than on those farther out.

This breakdown of pectic compounds more probably is a reaction to an enzyme. Many parastic fungi and bacteria produce pectinase, an enzyme which diffuses out ahead of the fungus ("acts in advance"), breaking down the middle lamellae of the host-cell walls and causing maceration of the host tissues.

Harter and Weimer (*9, 10, 11, 16*), in extensive studies on Rhizopus, have shown (*9, p. 623*) that *Rhizopus tritici* Saito "produces a powerful intracellular and extracellular pectinase," capable of macerating disks of raw sweet potato. A comparative study of several species of Rhizopus (*16, p. 132*) showed a marked variation in the amount of enzyme produced. It is almost absent (*11*) in *R. nigricans* Ehr., a parasitic species. The enzyme is produced at any temperature at which the fungus will grow.

If this reaction in the tissues about the infection is due to an enzyme from the fungus, it may be asked why the cells closest to the fungus are not affected most. Carré finds, from work on the pectic constituents of apples, that acids affect the activity of the enzyme, and suggests (*5, p. 837*) "that the enzymes effecting the decomposition of the pectic acid produced from the hydrolysis of the other pectic substances in the tissues can only operate when the acidity of the cell sap is lowered." It may be that within the mycelial area where host cells are dying, the breakdown of protoplasm gives rise to organic acids which check the activity of the enzyme, and that it can affect cell walls only when the enzyme has diffused out beyond the acids.

This reaction took place only in the case of infections on seedlings exposed to bright weather, a temperature range of 50° to 65° F., and a scant water supply. The infections in wet, dark weather, with a temperature range of 49° to 60° F., were nearly free from this decomposition of walls.

According to Dickson, Eckerson, and their associates (*6, 7, 8*), the composition of the cell walls of seedling wheat is influenced profoundly by the temperature of the soil in which it is grown. At high soil temperatures the walls are composed chiefly of pectic substances; at low temperatures the walls are principally cellulose. These writers find that susceptibility to the seedling-blight fungus, *Gibberella saubinetii* Durieu and Mont., is correlated with the character of the cell walls of the host. The fungus makes poor headway against cellulose walls, but penetrates pectic walls readily and apparently uses pectic materials as food. They find further that low soil moisture and low light intensity favor the development of the fungus.

Here, too, under the different weather conditions, there would appear to be a difference in the composition of the cell walls, perhaps due more to the extreme difference in soil moisture than to the slight difference in temperature. Little difference in susceptibility can be ascribed to it in this case, however, as the parasitism in rusts is of a very different type from that of Gibberella. Rust hyphae remain intercellular, and the host cells are entered only by haustoria, which seem to penetrate pectose and cellulose with equal ease. So far as is known, wall materials of the host are not used as food by cereal rusts.

It may be that this enzyme is produced in about equal amounts in all three experiments but that when the walls of the host are composed chiefly of cellulose the enzyme produces little disturbance. Only when, under different environmental conditions, the metabolism

of the host is altered and its cell walls contain more of the pectic compounds does the enzyme produce a conspicuous effect.

There is an alternative possibility. Harter and Weimer (*10*) have shown that in Rhizopus the substrate influences the production of pectinase. In several synthetic media lacking pectin, the enzyme is absent; when pectin is present, the enzyme is produced. It is possible that the production of pectinase is similarly regulated in this case.

A further fact should be considered. In studies of this same physiologic form of leaf rust on Little Club (*4*), a susceptible host, one set of rusted seedlings was grown beside the first set of Malakoff seedlings, inoculated April 30, 1924, and was subjected to the same temperature, bright weather, and scant water supply. There was no trace of decomposition of pectic wall materials in or about the infections on Little Club.

It may be, of course, that the composition of cell walls of Little Club leaves differs from that of Malakoff even when grown under parallel conditions. Dickson and Holbert (*7*) find such differences between varieties of corn. It also may be that the walls are the same and that the fungus contains the same enzyme. Muhleman (*13*) finds that under the conditions of his experiments the mycelium of Sclerotinia produces pectinase but that it does not excrete it into the culture media. Perhaps *Puccinia triticina* physiologic form 11 regularly produces pectinase but does not excrete it. In that case the enzyme would be released only when the fungus is damaged as on a resistant host.

The question is complicated by too many possibilities, and the exact knowledge is too scant to permit any final decision.

In all three lots of infections studied, the initial reaction of the host to the rust is one of extreme intolerance, and in all three such mycelia as survive the critical period of the first few days gain slowly in strength. Some of them live on for two or three weeks. The chemical interplay of fungous secretions and host reactions becomes regulated or altered or adjusted in some fashion so that the rust thrives to a greater degree.

It is not known how widespread this chemical interplay is. It has been observed before in infections of stem rust on Mindum wheat (*2*) and Khapli emmer (*3*).

The nature of this adjustment process is purely a matter of conjecture. The first haustorium evidently secretes some substance into the host cell which kills the cell. It may be that the fungus gradually stops secreting this substance, and so, by permitting the host cells to survive, can itself make better growth. Or, as has been suggested before (*3*, *p. 722*), the first secretion of this harmful substance may diffuse out slowly into adjoining host cells, setting up there some counter-reaction, perhaps in the nature of antibody formation, by which, when these cells in turn are invaded by haustoria, the harmful fungous secretion is neutralized. The host cell then survives, the rust has living cells from which to draw food, and if its own metabolism is not interfered with by the reactions of the host, a mode of living is established. If this supposition is correct it is, by an odd reversal of what one would expect, the defensive reaction of the host which renders it more susceptible, for this highly specialized parasite lives only when its host is able to survive.

The difference between the initial reaction and the final one is not great here. Graded on the usual scale of 0 to 4, even the best reaction would rank as 0+ or 1, for the few uredinia produced are small and often remain subepidermal. In other material, however, the spread between the initial and final reaction is greater (unpublished data). Some infections remain microscopic in size; others that succeed in surviving the critical stage may bear fair-sized uredinia. It may be that this is one of the factors operative in producing the "X" or "heterogeneous" reaction noted by Stakman and Levine. They define this reaction (*14, p. 5*) as follows: "Uredinia very variable, apparently including all types and degrees of infection on the same blade; no mechanical separation possible; on reinoculation small uredinia may produce large ones, and vice versa. Infection ill-defined." This adjustment process may be one of the factors, but it is almost certainly not the only one contributing to the production of the heterogeneous type of infection.

SUMMARY

Malakoff wheat is highly resistant to *Puccinia triticina* physiologic form 11 at all ages of the host and under all environmental conditions tested.

In the young infections the first host cells invaded collapse and die promptly. Many of the young rust fungi die at this stage. If they survive this first severe struggle for existence they gain slowly in strength. As successive host cells are entered by haustoria, the host reaction becomes milder and slower until finally a haustorium may form, expand partly, function for a short period, and undergo normal drainage. The rate of growth of the mycelium becomes correspondingly increased. A few of the mycelia bear small uredinia.

It is suggested that this adjustment process may be one of the factors operative in producing the "X" or "heterogeneous" type of infection described by Stakman and Levine.

In infections exposed to bright weather and scant water supply, a zone of host tissue surrounding the infection shows pronounced degradation of the pectic constituents of the cell walls. In seedlings and older plants inoculated during the dark wet weather of January and February this reaction is slight and sometimes absent.

This breakdown of pectic substances may be due to acids formed in the dying and decomposing host cells within the mycelial area, but it is more probably due to an enzyme from the fungus.

The difference in the amount of this pectic reaction in plants subjected to different environmental conditions may be due to a difference in the composition of the mesophyll cell walls brought about by those conditions.

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THE SWINE HERDBOOK AS A SOURCE OF DATA FOR THE INVESTIGATION OF THE SEX RATIO AND FREQUENCY OF SEX COMBINATIONS IN PIG LITTERS[1]

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INTRODUCTION

The vast number of pigs so far registered by the various breed associations in this country and others would be greatly in excess of the needs for a satisfactory statistical investigation of sex ratio and sex constitutions of litters were it not for the fact that in most cases the data are incomplete. When there is omitted from the record the sex constitution at birth of the litter to which the registered animal belongs, the record becomes worthless for any study in which sex is concerned. Some swine associations do not record the size of the litter, others record the size of the litter and the number of animals raised, while still others record the size of the litter and the sex of those raised. Examples of the last class are the Chester White and the National Duroc-Jersey Associations. But of the millions of litters registered by these two associations only a small percentage can be used for study because only those litters in which all the pigs were raised can be taken. Thus, the data become reduced to a relatively small amount.

Practically all the references to sex ratio and the frequencies of sex combinations in swine are based on herdbook data. Among the citations of sex ratio are those of Crew (*2, p. 255*),[2] who gives 111.8:100 and Rice (*6, p. 158*), quoting Wilkens, who gives 108.74:100. Parkes (*5*) reports 104.7:100. Carmichael and Rice (*1*) observed a ratio of 108.16:100. Wentworth (*7*), studying the frequency of sex combinations, found little divergence from probability. A similar result was obtained by Duncker (*3*). The work of the last two writers is incomplete, however, since only a few litter sizes were studied. A much more extensive study of the situation has been made recently by Parkes (*5*), who used data taken from the National Duroc-Jersey Record. Among 2,020 litters ranging in size from 2 to 14 and comprising 16,233 young he found the proportion of boars to be 0.4884. The frequencies of the sex combinations in the different litter sizes were found to diverge greatly from the calculated frequencies on the basis of the expansion of the binomial $N(p\,q)^n$, in which p is the mean proportion of boars, q the mean proportion of sows, N the number of litters, and n the size of the litter. Only in litters of five was there a reasonably close agreement between the observed and the calculated frequencies. The exceedingly poor fits seemed in general to be caused by an excess of litters in the mean sex combinations and a deficiency of litters in the extreme sex combinations. Such a condition is, as Parkes indicates, very difficult to explain on biological grounds.

[1] Received for publication Jan. 29, 1927; issued June, 1927.

[2] Reference is made by number (italic) to "Literature cited," p. 726.

MATERIAL

The present study is based on two entirely separate sources of data: (1) The records of the Chester White Swine Association, and (2) the compiled records of the experimental herds maintained by the United States Department of Agriculture at Beltsville, Md., and various field stations. The experimental data were used for the purpose of checking the herdbook data.

In the Department of Agriculture data all litters recorded, except those having one or more pigs of undetermined sex, were included. These data are accurate and for the purposes of the present study include size of litter, number of pigs born alive, sex of pigs taken at birth, and complete information on mortality to weaning at 10 weeks of age. It is thus possible to study sex ratio among stillborns as well as among those born alive and to determine whether there is differential mortality between birth and weaning. The pigs were distributed among the Poland-China, Duroc-Jersey, Tamworth, and Chester White breeds.

The Chester White Swine Record gives the name and number of each animal, date born, size of litter, the sex of those raised, and the names of the parents. The only items used in this study are size and sex constitution of litters. Furthermore, only litters in which all the pigs were raised could be included, since only in such litters was the sex of all pigs born given. The method used was to start at the beginning of volume 26 of the Chester White Swine Record and tabulate the sex constitution of all complete litters of which the registered boars were members, care being taken to avoid duplicate litters. This was continued until 1,541 litters had been tabulated. There was no reason for discontinuing the tabulation at this point except that it was considered to be sufficient.

It should be explained that in the front of the Chester White Swine Record only boars are registered and in the back only sows are registered. Thus the 1,541 litters which were tabulated were recorded because one of each litter was a registered boar. In such data it would be impossible to find a litter consisting of all sows, because in such a case, if the entire litter were registered, they would appear among the sows in the back of the volume. It was necessary to tabulate another 1,541 litters from that part of the record in which the sows are registered, thus doubling the number of litters for study, and giving an equal chance for the occurrence of unisexual litters. Volume 27 was used for obtaining the 1,541 litters from registered sows. The purpose in using a different volume was primarily to avoid taking litters which were duplicates of those taken from the boars.

It was found that the number of litters larger than 12 was very small and for purposes of detailed study only litters of 12 and less were included.

PROPORTION OF BOARS

Consideration will first be given to the proportion of boars found in the Chester White Record data. In Table 1 are shown the number of litters and the number and proportion of boars. The most noticeable feature of these data is the low proportion of boars which in all litters except 2, 3, and 5, is below 0.5000. The mean proportion comes out $0.4852 \pm .0022$. The mean proportion of boars found

by Parkes (*5*) in 2,020 litters of 2 to 14 taken from volume 47 of the National Duroc-Jersey Record was $0.4884 \pm .0026$. The difference between these two results is only $0.0032 \pm .0035$, a difference which certainly is not significant. Their average is $0.4865 \pm .0018$.

TABLE 1.—*Size of litters, number of pigs, and number and proportion of males in 3,082 litters from the Chester White Swine Record*

Size of litter	Number of litters	Number of pigs	Number of males	Proportion of males
1	0			
2	14	28	14	0.5000
3	72	216	109	.5046
4	116	464	207	.4461
5	226	1,130	574	.5080
6	360	2,160	1,074	.4972
7	452	3,164	1,543	.4877
8	712	5,696	2,744	.4817
9	472	4,248	2,039	.4800
10	410	4,100	1,989	.4851
11	178	1,958	943	.4816
12	70	840	410	.4881
Totals	3,082	24,004	11,646	.4852

There is reason for believing that the foregoing figures for proportion of boars are too low. Carmichael and Rice (*1*) report 0.5196 of males in a population composed of several breeds. Severson,[3] in a preliminary report, gives a mean proportion of $0.5230 \pm .0056$ males in a group of 3,779 pigs representing five pure breeds. The United States Department of Agriculture data, which are given in Table 2, show a mean proportion of $0.5199 \pm .0038$ males in 7,854 pigs, consisting of several pure breeds. None of these determinations differ statistically from one another. Their average comes out $0.5201 \pm .0026$ males among 17,290 pigs. The difference between this figure and $0.4865 \pm .0018$ for the herdbook material is $0.0366 \pm .0031$. Since this difference is almost 11 times its probable error there can be no doubt about its significance, and it seems probable that the proportion of males shown by the herdbook data is too low by about 3 per cent.

TABLE 2.—*Size of litters, number of pigs, and number and proportion of males in the litters of the Bureau of Animal Industry herd, 1921 to 1926*

Size of litter	Number of litters	Number of pigs	Number of males	Proportion of males
1	7	7	3	0.4286
2	17	34	22	.6471
3	32	96	51	.5313
4	43	172	97	.5640
5	44	220	122	.5545
6	74	444	240	.5405
7	98	686	364	.5306
8	106	848	439	.5177
9	137	1,233	635	.5150
10	107	1,070	555	.5187
11	85	935	490	.5241
12	60	720	361	.5014
13	46	598	308	.5151
14	28	392	205	.5230
15	11	165	79	.4788
16	7	112	56	.5000
17	5	85	40	.4706
18	1	18	9	.5000
19	1	19	7	.3684
Totals	909	7,854	4,083	.5199

[3] SEVERSON, A. PROLIFICACY OF SOWS AND MORTALITY OF PIGS. Amer. Soc. Anim. Prod. Proc. 1925/26, (In press.)

The low proportion of males shown by the herdbook probably is to be explained upon the basis of faulty data rather than upon any biological grounds. There are, of course, many ways in which errors could be introduced, either intentionally or unintentionally. A few of these possibilities may be considered briefly. From the very nature of the case, however, the means by which the defects were introduced can only be speculated upon.

Failures of the breeders to report stillborn pigs would affect the sex ratio in case the proportion of males among stillborns differs significantly from the average. Data collected by the Department of Agriculture show that the proportion of boars among the stillborns is $0.5639 \pm .0136$; Carmichael and Rice (*1*) report 0.5600. These figures are in excess of the proportion of males among those born alive by about 4 per cent and are in line with what would be expected on the basis of Crew's (*2*) findings relative to the effect of prenatal death on the sex ratio in swine. Crew reports a sex ratio of almost 150:100 at conception. This diminishes to nearly equality at birth and it follows that a much larger proportion of boars than sows is to be expected among prenatal deaths. Whether the omission of all stillborns would be sufficient to lower the proportion of boars to that which has been found to exist in the herdbook depends on the proportion of stillborns which occurs. The number necessary to lower the proportions of males in the Chester White herdbook from an assumed original 0.5320 at birth to the observed 0.4852, assuming a proportion of 0.5600 males among the stillborns, has been calculated and found to be the absurd number 20,894. That is, 44,900 pigs containing 52 per cent of boars at birth and having 20,894 stillborns, among which the proportion of boars was 0.56, would show a proportion of 0.4852 boars if the stillborns were omitted. Such a number of stillborns is 46.5 per cent of the theoretical original population. The percentage of stillborns observed in the Department of Agriculture herd was 7.7. Carmichael and Rice (*1*) report 9.3 per cent and Severson[4], 3.6 per cent. All these figures are from experimental herds and are not strictly comparable with the herdbook data, but they indicate that probably 7 per cent would be a fair estimate. This suffices to show that anything like the calculated 46.5 per cent is impossible. From the breeder's standpoint also it seems almost impossible that there should be a widespread custom of omitting stillborn pigs from the report filed with the breed association, although it is probably done by some individuals. It seems certain, then, that the omission of stillborns is not the decisive factor in bringing about the low proportion of boars observed in herdbook data.

There is a possibility that the differential mortality among the sexes may influence the proportion of boars observed in herdbook data. According to Crew (*2*) the male pig is less able than the female to withstand the stress of functioning both before and after birth. If this is true a litter having a high percentage of sow pigs should stand a better chance of being raised in its entirety than a litter having a high percentage of boars. For this reason it is possible that those litters which appear in the herdbook as entire litters have a slightly higher percentage of sow pigs than is representative

[4] SEVERSON A. Op. cit.

of the condition at birth in the whole population. No data are on hand, however, to prove the point.

Since in this study only litters in which all pigs were raised could be used, it is interesting to compare the frequency with which the different litter sizes occur with that shown by the experimental litters. Such a comparison is presented in Figure 1. In making this comparison the Department of Agriculture data have been rated up to the same total as the Chester White. However unsafe such a procedure may be, it gives rise to some interesting speculations as to what the breeder may have done which will affect the results reported in this paper.

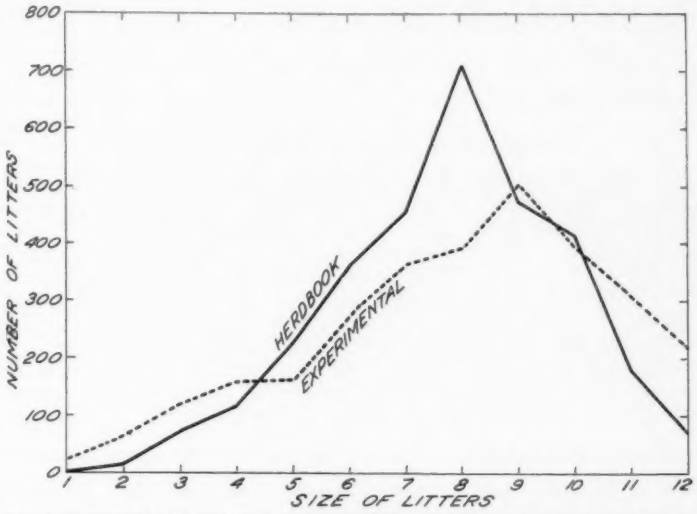

FIG. 1.—Frequency of different litter sizes as shown by herdbook data and by records of experimental herds. The total number of litters is the same in each lot. The herdbook litters are fewer than expected in the extreme sizes and far in excess of expectation in the medium sizes

It is seen that the herdbook data show fewer litters of 1 to 4 and of 9, 11, and 12 pigs, than do the experimental data. From this it may be inferred that fewer animals from small litters were registered than should have been to make the proportion of these litters representative. Among the larger litters it is not surprising, however, that there should be a deficiency, since only litters in which all pigs were raised have been used, and it is a matter of common knowledge that as litters increase in size beyond five or six the difficulties of avoiding mortality in raising the pigs become greater. However, since all the pigs are supposed to have been raised, this factor should not affect the present data. The frequency of litters of 8 in the herdbook data is almost double that in the experimental data. This fact is difficult to explain on any other ground than a misinterpretation of litter size; that is, the litters which are reported as "8, all raised," are probably in some cases the remainders of larger litters or combinations of two or more small ones. Just how such a hit-and-miss procedure could lower the sex ratio is not clear, however,

since Parkes (*5*) has shown that there is only a very low negative correlation between size of litter and proportion of boars.

It should be kept in mind that the herdbook data used included only litters of 2 to 12 and 2 to 14, while the experimental data include litters ranging from 1 to 20. The omission of the larger-sized litters from the herdbook material could not lower the proportion of boars through the relation between sex and size of litter, however, because the correlation is a negative one and the tendency would be to raise the proportion.

In conclusion, it may be said that so far as the sex ratio is concerned the herdbook data give a ratio which is significantly less than that given by a large body of experimental data. The reasons why the sex ratio is lower in the herdbook data are not clear, but indications point most strongly to errors introduced in reporting and recording the sex of the pigs. There is no indication that the condition has any biological explanation.

FREQUENCY OF SEX COMBINATIONS

In swine, where multiple births are the rule, a variety of sex combinations may occur. The number of different possible combinations in any litter size is given by the formula $n+1$, in which n is the size of the litter. If sex determination is a random process, the distribution of such sex combinations in each litter size should be given by the expansion of the binomial $N(p+q)^n$, in which N is the number of litters of any given size, n is the number of pigs in the litter, and p and q are the proportions of males and females, respectively.

In testing the closeness of agreement between the observed frequencies and those given by the binomial expansion, use can be made of Pearson's chi-square test for goodness of fit. The ordinary formula for calculating χ^2 is $\chi^2 = S\left(\frac{x^2}{m}\right)$, where m is the expected number and x is the difference between expected and observed. Extensive tables are available which give the values of P for the different values of χ^2 and n. The method gives a value for P which shows the probability in future trials of obtaining a greater value for χ^2 than the one observed. Its use in this paper is to determine whether the differences between the observed and calculated values are caused by sampling or by factors affecting the distribution of sex frequencies. It is obvious that as the value of χ^2 increases for any value of n the value of P decreases. It becomes necessary, therefore, to select some arbitrary range in the scale of values within which it may be said that the value of P indicates an unquestioned agreement between observed and calculated values. According to Fisher (*4*) such a range is from 0.1 to 0.9. That is, if it is assumed as a working hypothesis that the frequencies of sex combinations in the different litter sizes are given by the expansion of the binomial $N(p+q)^n$, a value for P of something less than 0.1 must be obtained before the hypothesis can be called in question and a value of less than 0.05 must be obtained before it is a certainty that the hypothesis does not fit the observed values.

TABLE 3.—*Values of P for testing goodness of fit in Department of Agriculture, Chester White Swine Record, and National Duroc-Jersey Record data*

Size of litter	Department of Agriculture data	Chester White Swine Record	National Duroc-Jersey Record
2	0.1952	0.8900	0.7303
3	.9665	.3926	.7314
4	.6831	.0481	.2538
5	.2623	.3433	.8277
6	.6906	.0221	.3366
7	.9160	.7707	.3192
8	.8378	.0002	.0001
9	.4995	.0000	.0759
10	.6678	.0054	.0017
11	.8665	.0197	.2112
12	.1652	.0125	.1298

In Table 3 are shown the values of χ^2 and P for testing agreement between the observed and calculated frequencies of sex combinations in litters of 2 to 12, inclusive, of the Department of Agriculture, Chester White Record, and National Duroc-Jersey Record data. The calculated and observed frequencies in the Department of Agriculture and Chester White Record data are also shown in Tables 4 and 5, respectively. If Fisher's (*4*) range in values for P is taken as indicating agreement between observed and calculated, it may be said that in litters of 4, 8, 9, 10, 11, and 12 of the Chester Whites and litters of 8, 9, and 10 of the Duroc-Jerseys, the observed frequencies show such marked divergence from the calculated as to indicate either a serious fallacy in the hypothesis or faults in the data. In view of the fact that herdbook data give good fits in some litter sizes, it seems probable that the data are at fault. This becomes more apparent when the values of P for the Department of Agriculture data are examined. It is seen that the range in values of P for the experimental data is from 0.165 to 0.966, and it may be said that such values lie within the expected range. It is particularly important to note that in litters of 8, 9, and 10 the values of P are 0.8378, 0.4995, and 0.6678, respectively, thus showing good fits. Since it is in these three litter sizes that the herdbook data show exceedingly wide departures from expectation, this result becomes very important, indicating that there is a very serious defect in this portion of the herdbook data. In view of the fact that it was in these same litter sizes that a considerable discrepancy has been noted between the numbers of experimental and herdbook litters, it seems desirable to analyze the situation further.

TABLE 4.—*The observed and calculated frequencies of sex combinations, together with value for goodness of fit for Department of Agriculture experimental data; calculated on the basis of mean proportion of males*

Number of males	2		3		4		5		6		7		8		9		10		11		12	
	Observed	Calculated	Observed	Calculated	Observed	Calculated	Observed	Calculated	Observed	Calculated	Observed	Calculated	Observed	Calculated	Observed	Calculated	Observed	Calculated	Observed	Calculated	Observed	Calculated
0	1	3.9	3.0	3.5	2	2.6	1	1.2	1	0.9	0	0.6	0	0.3	0	0.2	0	0.1	0	0	0	0
1	10	8.5	12.0	11.5	8	11.3	4	6.3	4	5.9	4	4.4	5	2.6	3	1.9	1	.8	0	.3	0	.1
2	6	4.6	12.0	12.5	15	12.2	12	13.7	13	15.9	12	13.3	9	9.8	5	8.3	6	3.7	1	1.7	3	.7
3			5.0	4.5	13	13.3	13	14.8	28	23.0	30	25.9	22	21.3	20	21.0	5	10.6	3	5.6	2	2.7
4					5	3.6	12	6.3	17	18.7	25	28.0	25	28.8	36	30.1	21	20.1	14	12.1	8	6.5
5							2	1.7	8	8.1	18	18.2	26	24.9	38	32.6	30	26.1	19	18.3	10	10.2
6									3	1.5	8	6.6	14	13.5	21	26.7	22	23.6	21	19.8	13	12.9
7											1	1.0	4	4.2	10	12.4	14	14.6	17	15.3	11	12.0
8													1	.6	4	3.6	7	5.9	7	8.3	8	8.9
9															0	.4	1	1.4	3	3.0	2	4.3
10																	0	.2	0	.6	3	1.4
11																						.3
Total	17	17.0	32	32.0	43	43.0	44	44.0	74	74.0	98	98.0	106	106.0	137	137.2	107	107.1	85	85.0	60	60.0
X^2	0.1687		3.3178		2.2960		6.5129		3.8946		2.0330		3.4484		6.3632		5.8317		3.1857		11.7226	
X	0.1952		0.9645		0.6831		0.2623		0.6906		0.9160		0.8378		0.4995		0.6678		0.8655		0.1652	

TABLE 5.—*The observed and calculated frequencies of sex combinations, together with value for goodness of fit for Chester White herdbook data; calculated on the basis of mean proportion of males*

Number of males	2		3		4		5		6		7		8		9		10		11		12	
	Observed	Calculated	Observed	Calculated	Observed	Calculated	Observed	Calculated	Observed	Calculated	Observed	Calculated	Observed	Calculated	Observed	Calculated	Observed	Calculated	Observed	Calculated	Observed	Calculated
0	3	3.7	6	9.8	8	8.1	8	8.2	1	6.7	1	4.2	2	3.5	2	1.3	0	0.5	0	0.1	0	0
1	8	7.0	29	27.8	44	30.7	29	38.5	30	37.9	26	28.6	22	26.5	3	10.8	4	5.1	0	1.2	0	.3
2	3	3.3	31	26.2	37	43.4	76	72.6	99	89.3	87	80.8	71	87.4	23	40.7	19	21.4	9	5.9	0	1.5
3			6	8.2	19	27.3	65	68.4	120	112.2	126	126.9	165	164.7	86	89.5	38	53.9	10	16.5	8	4.7
4					8	6.4	42	32.2	74	79.3	118	119.8	245	194.0	177	111.5	111	88.8	37	31.2	7	10.0
5							6	6.1	26	29.2	73	67.7	151	146.3	100	105.1	102	100.5	42	41.2	18	13.8
6									10	4.7	19	21.3	44	68.9	51	75.0	87	78.9	45	38.8	16	15.1
7											2	2.8	10	18.5	25	30.3	40	42.5	20	26.1	7	12.2
8													2	2.2	2	7.1	4	15.0	10	12.3	7	7.9
9															3	.7	5	3.1	4	3.9	3	3.3
10																	0	.3	0	.7	4	1.0
11																			1	.1	0	.2
12																					0	0
Total	14	14	72	72	116	115.9	226	226	360	359.3	452	452.1	712	712.0	472	472	410	410	178	178	70	70
X^2	0.3026		2.9950		9.6303		5.6675		14.061		4.0754		30.9654		73.5811		21.7789		18.2322		17.8951	
P	0.8900		0.3926		0.0481		0.3433		0.0221		0.7707		0.0002		0.0000		0.0054		0.0197		0.0125	

The standard deviations which are shown in Table 6 indicate that grouping into certain sex combinations is an important factor in bringing about the condition which has resulted in such marked disagreement between observed and calculated values. In the Chester White data the observed standard deviations are less than the calculated in all litter sizes studied except 4, 7, and 12. Parkes (*5*) found that in the Duroc-Jersey herdbook data the observed standard deviations were less than the calculated except in litters of 11 and 12. His figures are included in Table 6 for the purpose of comparison. Now it is important to note that although these differences exist, not all of them are of such magnitude as to be significant statistically. Among the Chester Whites only in litters of 8, 9, and 10 are the differences between the observed and the calculated standard deviations as much as or more than three times the probable error, and therefore only in these three litter sizes can it be stated definitely that we are dealing with differences, which are not caused by the fluctuations of random sampling. Likewise among the Duroc-Jerseys it is in litters of 8, 9, and 10 that the greatest differences are observed, and although Parkes (*5*) does not give the probable errors, an examination of the magnitude of the differences will show that only in these three litter sizes are the differences significant. Then it may be said that the two groups of data are essentially similar in this one respect. Among the experimental litters, on the other hand, the situation seems to be different in that there are no significant differences between the observed and the calculated standard deviations. Furthermore, there seems to be no regularity with respect to the cases in which the observed is less than the calculated.

TABLE 6.—*Observed and calculated standard deviations for frequency of sex combinations in each litter size of Department of Agriculture, Chester White Swine Record, and National Duroc-Jersey Record data*

Size of litter	Department of Agriculture data		Chester White Swine Record		National Duroc-Jersey Record	
	Observed σ	$\sqrt{npq}$	Observed σ	$\sqrt{npq}$	Observed σ	$\sqrt{npq}$
2	0.5703	0.6758	0.6547	0.7071	----------	----------
3	.8609	.8643	.7636	.8659	----------	----------
4	1.0421	.9918	1.0239	.9941	0.824	0.999
5	1.1272	1.1114	1.1173	1.1178	1.069	1.118
6	1.2171	1.2207	1.1737	1.2247	1.103	1.224
7	1.2952	1.3204	2.7574	1.3224	1.253	1.303
8	1.4886	1.4133	1.2502	1.4132	1.194	1.414
9	1.4492	1.4993	1.3257	1.4636	1.350	1.499
10	1.5562	1.5799	1.4415	1.5804	1.430	1.581
11	1.4966	1.6564	1.6037	1.6541	1.688	1.658
12	1.9018	1.7320	1.8615	1.7315	1.769	1.731

What do these conditions mean with respect to the departure from expectation in distribution of the different sex combinations? First of all, a grouping into the mean sex combinations is clearly evident in both groups of herdbook data and just as clearly absent from the experimental data. From the experimental data we observe that the frequencies of the different sex combinations are essentially those given by the expansion of the binomial $N(p+q)^n$. In litters of 8, 9,

and 10 of herdbook data this relation does not hold, however, and reference to Table 5 will reveal the reason why. There is a noticeable surplus of litters in the mean sex combinations and a deficiency of litters in the extreme sex combinations. Such a condition can come about only through a failure to report the actual condition at birth. The tendency has been to report a large proportion of the litters as containing an equal number of males and females, which fact strongly suggests a too extensive use of the human memory as a record book. The causes which limit the deficiencies to litters of 8, 9, and 10 are probably economic ones. First of all, these particular litter sizes are among those which occur most frequently, and hence it may be expected that they would offer the greatest opportunity for the introduction of irregularities. Secondly, the sizes are such as to be easily synthesized by combining two smaller litters and registering as one. This would account for at least a part of the excess of litters of 7 and 8, and the deficiency of litters of 2, 3, and 4 in the herdbook data. Of course certain litters born as 12 or more and in which only 8 or 9 were raised might be registered as the number raised. There are other possibilities as well, but little is to be gained from discussing them. One thing seems to stand out very clearly, that the process of introducing the defects is essentially as complicated as human nature, and hence the possibility of rectifying the errors by a process of correction is not only remote but practically impossible. This is exceedingly regrettable because the only value which swine herdbook data have or probably ever will have for research purposes consists of that which pertains to the sex of the pigs and the size of the litter. There can be little doubt that in both these respects the data are too unreliable to be of any real value.

In this connection it may be well to lay particular emphasis on the point that the results reported in this paper concerning the value of herdbook data for investigational purposes apply to swine herdbook data in general and not to those of any one breed. The defects appear to be of the same general nature in both the Chester White and the Duroc-Jersey breeds. In other words, the fact that the Chester White Swine Record has been used in the present study and has been found to be defective in certain respects should not be interpreted as meaning that the Chester White Swine Record is any more inaccurate or incomplete than are the records of the other breeds.

SUMMARY AND CONCLUSIONS

The proportion of males in swine at birth as shown by three lots of independent experimental data is approximately 0.52.

The proportion of males found in herdbook data is too low by between 3 and 4 per cent.

In the experimental data the frequency with which the different sex combinations in each litter occurred are in agreement with those given by the expansion of the binomial $N(p+q)^n$, in which N is the number of litters and n the size of the litter.

Herdbook data do not possess the necessary accuracy for a study of sex ratio and frequency of sex combinations. The most apparent defects seem to be a failure to report and record accurately the sex of pigs and the size of litter at birth. These defects are not peculiar to the records of any one breed association.

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RELATION OF SIZE OF OIL DROPS TO TOXICITY OF PETROLEUM-OIL EMULSIONS TO APHIDS[1].

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INTRODUCTION

Petroleum oils, largely in the form of miscible oils or of emulsions, are used to control scales and other insects on fruit trees. A miscible oil contains a dissolved emulsifier, which causes emulsification when the oil is properly diluted with water. Sulphonated oils, the material obtained in the refining of petroleum, and similar emulsifiers are soluble in oil. Others, such as soap, are made to dissolve in oil by the addition of a third substance like cresylic acid. Usually the miscible oils contain a little water and are clear and oily in appearance.

Some miscible oils mix readily with water when poured directly into it. Others must have a little water stirred into them before they can be further diluted. If water is poured directly into a miscible oil of the second type an inverted or water-in-oil emulsion, which floats on the surface of the water, is formed. When properly diluted, however, miscible oils of either type form milky emulsions in which the oil drops are very small, usually less than 2 microns in diameter. Such diluted emulsions cream out slowly on standing, and are very resistant to breaking by hard water.

The oil drops in oil emulsions are usually larger than those in diluted miscible oils. The size depends on the emulsifier used and on the method of preparation. Oil emulsions, when diluted with water, cream much more rapidly than the emulsions resulting from the dilution of miscible oils. Those made with a soap emulsifier are much less resistant to breaking in hard water than are diluted miscible oils.

It has been the usual experience that a miscible oil diluted for spraying requires a larger percentage of oil than a lubricating-oil emulsion. Miscible oils for dormant spraying are generally used at a dilution of about 1 gallon to 15 gallons of water and contain from 80 to 90 per cent of petroleum oil. At the 1 to 15 dilution they would have an oil concentration of approximately 5 per cent. Lubricating-oil emulsions for dormant work have usually had a concentration of 2 or 3 per cent oil. This discrepancy in dosage has probably sometimes resulted from the use of oils lighter and less toxic than the lubricating type. No reports of experiments with miscible oils and emulsions made from the same petroleum oil have been found in the literature. Jarvis (*6*)[2] and Houghton (*5*), who used a paraffin oil of lubricating type in some of their preparations, showed that from 5 to 10 per cent of oil was necessary for satisfactory control of the San Jose scale.

[1] Received for publication Jan. 8, 1927; issued June, 1927.
[2] Reference is made by number (italic) to "Literature cited," p. 737.

The main differences between a diluted miscible oil made with cresylic acid and soap and a diluted soap emulsion appear to be the presence in the former of cresylic acid and the difference in the size of the oil drops. It is conceivable that either of these factors might cause a difference in toxicity.

The cresylic acid might tend to lower the toxicity by either of two methods. First, it has been shown (*3*) that a petroleum oil-soap emulsion consists of droplets of oil, each surrounded by a film of soap and suspended in an aqueous medium. The cresols have a tendency to form molecular compounds or addition products with many organic compounds. They might form such compounds with the soap in the films surrounding the oil droplets, resulting in tougher films, which would prevent the oil from readily coming in contact with the surface of the plant being sprayed or with the insect to be killed. Secondly, it is conceivable that cresylic acid, in small proportions, might act physiologically on the insect, making it less susceptible to the toxic action of the oil.

Recently de Ong (*1*) has carried out studies which indicate that the permanent or stable type of emulsion is not so efficient for insecticidal purposes as a quick-breaking form—that is, one in which the oil separates out very quickly from the dispersed phase in the water. He suggests that the ideal form of insecticidal emulsion is one which approaches the mechanical type of emulsion, in that the droplets of oil are comparatively large and separate from the water almost immediately on striking the leaf surface.

The permanence or stability of an emulsion is very closely linked with its drop size, the emulsion with small oil drops being most permanent. This suggests that the lower toxicity of the miscible oils might be due to the small drop size as compared with oil emulsions.

When a spray is applied to a plant some of the liquid runs or drains off. The oil in the portion which drains off is lost. Only the portion which remains fixed is of value as an insecticide. The spray which drains off may contain a higher or lower percentage of oil than that first applied, depending on whether the oil or the water is more readily absorbed by the plant surface.

Moore (*7*) states that the electrical charge exhibited by a leaf surface (and presumably by most other external plant structures) should be negative because filter paper, consisting of cellulose, is negative, and cotton, which is chiefly cellulose, is also negative. He confirms this conclusion by showing that stains which give good results with leaf tissues are positive. As they are readily absorbed by the leaf tissue, that tissue must be negatively charged. To definitely settle the question he carried out endosmosis experiments with bean and citrus leaves. When a leaf diaphragm was placed between the two electrodes and the current was turned on, water migrated through the leaf in the direction of the negative electrode, showing that the leaf surfaces when wet bore a negative electrical charge.

The charge (*2*) on the suspended droplets of oil in an oil emulsion is usually negative. This negative charge would tend to prevent the oil from adhering to the negatively charged leaf or other similarly charged plant surface. The charge is naturally a surface phenomenon, and, other things being equal, it will be proportional to the area present. The area per unit volume of any droplet is its area

divided by its volume, or, as the oil droplets are spheres or nearly spheres, $\frac{4\pi r^2}{4/3\,\pi r^3}=\frac{3}{r}$, r being the radius. Thus the surface, and also the electrical charge associated with any volume of oil, are inversely proportional to the radius of the droplets into which the oil is divided. When the oil is dispersed into droplets with a radius of 0.5 micron, 10 times the charge is associated with the same volume of oil as when it is dispersed into droplets with a radius of 5 microns. This leads to the conclusion that the forces which tend to keep the oil from adhering to plant surfaces must be much greater in an oil dispersed in small droplets than in one dispersed in large droplets. Consequently, the proportion of oil held by a plant surface should be lower when the droplets are small, making the emulsion less toxic to insects than an emulsion with large oil droplets.

Experiments were undertaken to determine whether the toxicity of petroleum oils to insects could be correlated with the size of the oil droplets and, if so, to ascertain the reason.

EXPERIMENTAL PROCEDURE

MATERIALS

Ten petroleum oils, ranging from a light, volatile oil of low viscosity to a lubricating oil of high viscosity, and representing both the paraffin and naphthene base oils, were used. Their properties are given in Table 1.

TABLE 1.—*Properties of the experimental oils*

Sample No.	Oil	Base	Color	sity, 20° C.	Flash point	Fire point	Volatility (4 hours at 105° C.)	Viscosity (Saybolt, 100°F.)	Unsulphonated
				Grams per c.c.	° F.	° F.	*Per cent*	*Seconds*	*Per cent*
1	Kerosene		Light straw	0.811	130	150	58.2	31	83
2	Gas oil		Black	.879	170	205	23.3	50	64
3	Red engine oil (IV)	Paraffin or mixed.	Red	.902	400	455	.2	231	61
4	Liquid petrolatum	Paraffin (?)	White	.879	390	425	.2	218	97
5	Paraffin oil 115	Paraffin	Yellowish brown.	.883	360	400	.4	110	67
6	Paraffin oil 222	do	Reddish brown	.896	380	425	.3	218	68
7	Paraffin oil 318	do	Yellowish brown.	.897	415	475	.06	313	76
8	Naphthene oil 100	Naphthene	do	.912	305	335	1.6	105	63
9	Naphthene oil 202	do	Reddish brown	.919	335	365	.7	200	60
10	Naphthene oil 305	do	Brown	.924	345	380	.6	298	60

Sample 3 was a commercial red engine oil, probably a mixed base oil, that has been widely used to control insects on citrus and deciduous fruit trees. Sample 4 was a well-known brand of white medicinal oil. The viscosities of samples 5, 6, 7, 8, 9, and 10 (lubricating oils) were approximately 100, 200, and 300 seconds for each base (paraffin and naphthene).

The soap used was a commercial fish-oil soap of potassium base, having the following composition: Water, 62.8 per cent; fatty anhydrides, 28.5 per cent; potassium oxide, 5.8 per cent; glycerol, etc. (by difference), 2.9 per cent. The cresol was of United States Pharmacopoeia grade.

The size of the oil droplets was determined under the microscope by means of an eyepiece micrometer. A drop of dilute sodium hydroxide solution was added to prevent the agglutination of the droplets. These measurements are intended to give only the approximate average or usual size of the droplets, but are considered sufficiently accurate for the purposes of this investigation.

The emulsions were prepared by the following methods:

(1) A soap-oil emulsion was made either by the Government formula (*9*) or by the cold-mixed method (*8*). In the Government process a mixture in the proportions of 2 gallons of oil and 1 gallon of water, containing 1 quart of potassium fish-oil soap, was heated to incipient boiling, and then forced three times through a disk-type spray nozzle at a pressure of 40 pounds per square inch. In the cold-mixing process the oil was stirred into the soap in the same proportions, and water was then added. The usual drop size in either case was about 8 to 10 microns. A part of the emulsion obtained by one of these methods was passed several times through a colloid mill, the usual diameter of the drops in the resulting emulsion being about 2 microns.

(2) An emulsion was prepared by stirring cresol into potassium fish-oil soap, and stirring the oil into the resulting mixture (*8*). The proportions used were 2 gallons of oil, 1 quart of fish-oil soap, 8 fluid ounces of cresol, and 5 pints of water. The usual diameter of the drops in this emulsion was 2 microns or less. An emulsion containing the same ingredients in like proportion, but having drops of a diameter of 8 to 10 microns, was obtained by diluting the cold-stirred emulsion of soap and oil with water containing 0.5 per cent of cresol.

(3) An oil-water emulsion was prepared by passing a mixture of approximately 1 part of oil and 10 parts of water several times through a colloid mill. After the grinding the oil was largely in droplets about 10 microns in diameter. The emulsion so obtained was tested against the insects. A portion of this emulsion was allowed to stand for 24 hours. It had then separated into two layers, the droplets in the lower layer being mainly 2 microns or less in diameter.

Thus three pairs of preparations, the members of each pair having the same chemical composition, but differing in drop size, were obtained.

The foregoing proportions were taken in making all the preparations, but slight variations due to the adherence of the oil to the walls of the glassware used in measuring, to temperature changes, to foaming, and to other causes occurred. The approximate oil concentration (per cent by volume) was determined in each emulsion after preparation by a modified Babcock method (*4*). The dilutions used in the tests on aphids were made on the basis of these determinations.

SPRAYING METHODS

Aphis rumicis L., reared on dwarf nasturtium plants (*Tropaeolum majus*) in a greenhouse, was employed for these experiments. For each experiment portions of plants holding groups of aphids of all ages were used, the stems being immersed in water in a bottle. The plants were thoroughly sprayed, the surplus drops of liquid being removed by gentle shaking. The bottle with its contents was then

placed upon a sheet of white paper, the edges of which were coated with adhesive material to prevent the escape of living aphids. The spraying was done with an atomizer, under an air pressure of 5 pounds per square inch delivered from a compressed air line through an adjustable reducing valve. The percentage of dead aphids was determined at the end of 18 to 26 hour periods. In most instances, however, the time was from 20 to 24 hours, which is much longer than the period necessary to produce the maximum number of deaths at the concentration used. The temperature and humidity were not controlled.

At the conclusion of each experiment the aphids were counted and the percentage of dead individuals was determined. Three experiments were made for each concentration of oil. From 802 to 1,939 aphids were used for each set of three experiments, the number falling below 1,000 in only 4 of the 38 sets of experiments. The weighted mean percentage of dead aphids was determined for each set of experiments, and from this the mean deviation (per cent) was calculated. This simple method of evaluating deviation was considered sufficiently exact for the purposes of this investigation.

EXPERIMENTAL RESULTS

TOXIC EFFECT OF DILUTED EMULSIONS

The action of the petroleum-oil emulsions upon *Aphis rumicis* is shown in Table 2, and in Figures 1 and 2.

TABLE 2.—*Toxic action of oil emulsions on Aphis rumicis*

Preparation No.	Oil	Emulsifier	Method of emulsification	Size of oil drops	Concentration of oil by volume	Total number of aphids (3 experiments)	Aphids killed	Mean deviation of percentage killed
				Microns	*Per cent*		*Per cent*	*Per cent*
112	Kerosene	Potassium fish-oil soap	Hot, 40-pound pressure	8	1.0	1,050	96.7	±3.60
				8	2.5	962	99.2	±.35
77, 146	do	Potassium fish-oil soap and cresol	Cold-stir method	4	1.0	1,533	41.9	±15.98
				4	2.5	1,168	89.7	±6.57
147	do	Potassium fish-oil soap	Mixed by cold-stir method; ground in colloid mill	2	1.0	1,376	20.3	±9.64
116	Gas oil	do	Hot, 40-pound pressure	7-8	.5	1,036	85.3	±4.78
				7-8	1.0	1,175	98.5	±.73
119	do	Potassium fish-oil soap and cresol	Cold-stir method	2	1.0	1,219	54.7	±6.71
				2	2.5	1,430	92.5	±.91
148	Red engine oil IV	Potassium fish-oil soap	do	10	.5	1,248	92.3	±3.44
151	do	do	Cold-stir method; cresol added on dilution	10	.5	1,266	90.3	±7.92
152	do	do	Prepared from 148 by grinding in colloid mill	2	.5	1,735	46.5	±4.49
154, 155	do	None	Oil and water ground in colloid mill	8	.5	*1,939	89.5	±1.02
154-a	do	do	do	2	.5	1,505	70.6	±8.44
113	Liquid petrolatum	Potassium fish-oil soap	Hot, 40-pound pressure	8-10	.5	1,053	97.3	±1.68
74, 145	do	Potassium fish-oil soap and cresol	Cold-stir method	2	.5	1,149	61.5	±12.94
				2	1.0	1,128	93.6	±1.79
				2	1.5	1,189	96.0	±2.47
133	Paraffin oil 115	Potassium fish-oil soap	Hot, 40-pound pressure	8-10	.5	1,348	97.0	±2.14
139	do	Potassium fish-oil soap and cresol	Cold-stir method	2	.5	1,225	77.8	±1.87
134	Paraffin oil 222	Potassium fish-oil soap	Hot, 40-pound pressure	8-10	.5	1,250	99.2	±.95
140	do	Potassium fish-oil soap and cresol	Cold-stir method	2	.5	1,259	57.2	±13.80
156	do	Potassium fish-oil soap	Cold-stir method; ground in colloid mill	2-3	.5	1,164	71.7	±7.36
157	do	do	Cold-stir method; cresol added on dilution	8-10	.5	1,297	94.7	±3.21
158	do	None	Oil and water ground in colloid mill	10	.5	1,170	94.6	±3.69
158-a	do	do	do	2	.5	935	74.9	±3.32
135	Paraffin oil 318	Potassium fish-oil soap	Hot, 40-pound pressure	8-10	.5	1,330	98.0	±1.30
141	do	Potassium fish-oil soap and cresol	Cold-stir method	2	.5	1,371	55.7	±7.52
138	Naphthene oil 100	Potassium fish-oil soap	Hot, 40-pound pressure	8-10	.5	1,257	98.6	±1.25
144	do	Potassium fish-oil soap and cresol	Cold-stir method	2	.5	1,468	65.6	±6.13
137	Naphthene oil 202	Potassium fish-oil soap	Hot-40-pound pressure	8-10	.5	1,174	97.5	±2.07
143	do	Potassium fish-oil soap and cresol	Cold-stir method	2	.5	1,367	60.3	±8.54
159	do	Potassium fish-oil soap	Cold-stir method; ground in colloid mill	2	.5	892	80.7	±11.30
161	do	do	Cold-stir method; cresol added on dilution	10-12	.5	802	98.7	±1.25
160	do	None	Oil and water ground in colloid mill	10	.5	1,063	95.9	±3.47
160-a	do	do	do	2	.5	1,334	55.9	±3.10
136	Naphthene oil 305	Potassium fish-oil soap	Hot, 40-pound pressure	8-10	.5	1,064	98.7	±.59
142	do	Potassium fish-oil soap and cresol	Cold-stir method	2	.5	1,373	61.0	±5.25

* 4 experiments.

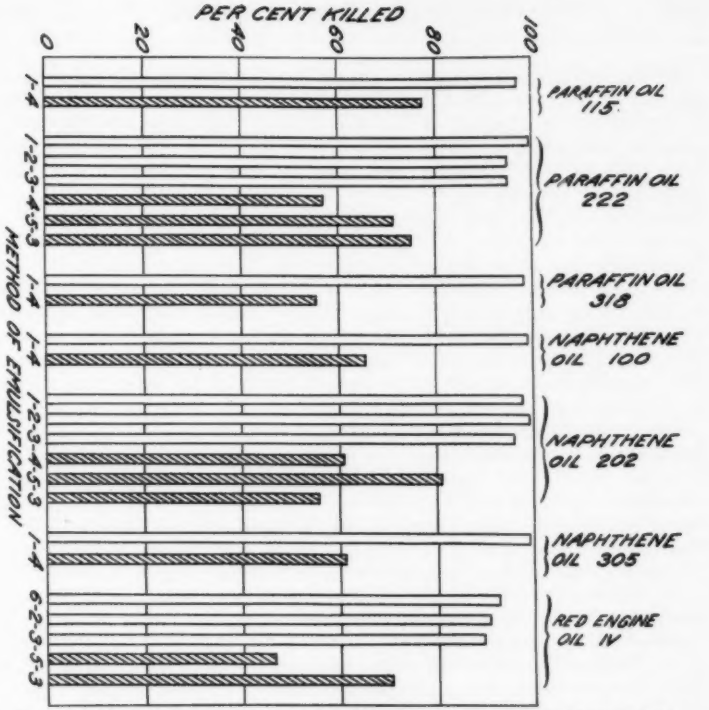

FIG. 1.—Toxicity to *Aphis rumicis* of lubricating-oil emulsions containing 0.5 per cent oil, showing the comparative effect of large and small drop size. Crosshatching indicates small oil drops

Method of emulsification: (1) Boiled soap oil-emulsion; (2) cold-mixed emulsion without cresol, cresol added on dilution; (3) oil and water ground in colloid mill, no soap; (4) cold-mixed emulsion with cresol; (5) cold-mixed emulsion without cresol, ground in colloid mill; (6) cold-mixed emulsion without cresol.

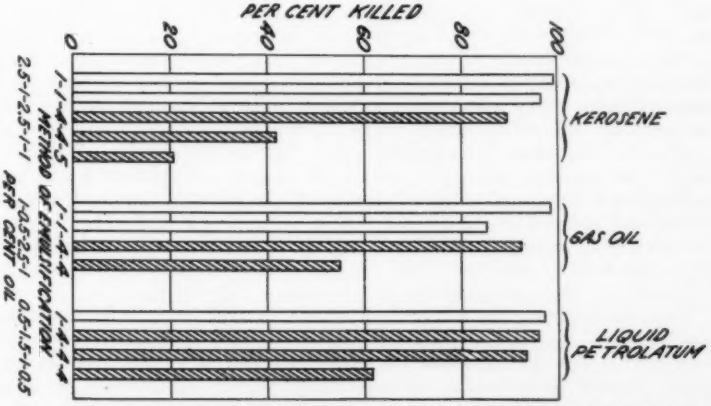

FIG. 2.—Toxicity of light and heavy oils to *Aphis rumicis*, showing effect of concentration and drop size. Crosshatching indicates small oil drops

Method of emulsification: (1) Boiled soap-emulsion; (4) cold-mixed emulsion with cresol; (5) cold-mixed emulsion without cresol, ground in colloid mill.

When the diameters of the drops lay between 8 and 12 microns, the oils of the lubricating type killed 90 to 100 per cent of the aphids at an oil concentration of 0.5 per cent. This was true for oils of paraffin and naphthene base, for oils ranging in viscosity (Saybolt) from 105 to 313 seconds, and for oils that contained a variable quantity of constituents reactive with sulphuric acid. (Table 1.) The method of preparation of the emulsion had little influence on toxicity (red engine oil IV, paraffin oil 222, and naphthene oil 202). The results obtained with red engine oil IV in an emulsion in which the droplets were large are slightly lower than the toxicity figures for the other high-viscosity oils. This result is probably not characteristic for emulsions of this oil in which the drop size is large, for some other preparations made with this oil, and not included in this investigation, have given higher toxicity at the same oil concentration.

When the drop size is small (2 or 3 microns or less in diameter), the toxicity of the emulsions containing lubricating oils is significantly lowered, the ratio of aphids killed falling within the range of 40 to 80 per cent. Table 1 and Figure 1 show that this low toxicity persists, regardless of the base, viscosity, or other physical characteristics of the oil, and that it is not altered by the method of preparation of the emulsion. That the presence of cresol does not influence toxicity, either directly by its action upon the aphids or indirectly through some effect upon the emulsion, is also brought out by these experiments. The emulsions of oil and water mixed by grinding in the colloid mill gave toxicities that fall within the range of the toxicities produced by emulsions containing cresol, the drop size in both preparations being about 2 microns. Furthermore, when cresol is added to emulsions of large drop size (8 to 12 microns), their toxicities are not appreciably changed.

The difference in toxicity between the emulsions of kerosene and of gas oil containing relatively large oil droplets (7 to 8 microns in diameter) and those containing relatively small droplets (2 to 4 microns in diameter) is clearly indicated in Table 2 and Figure 2. In experiments with 1 and 2.5 per cent kerosene, the emulsion having been prepared by the cold-stir method with cresol, the drop size was larger than usual (4 microns). This may account for the relatively high toxicity (89.7 per cent) obtained with a concentration of 2.5 per cent of this oil as compared with the toxicity from an emulsion applied at the same oil concentration, but in which the oil droplets were 8 microns in diameter. A similar relation is shown by a comparison of a kerosene emulsion, made by the cold-stir method without cresol and ground in the colloid mill to a drop size of 2 microns, with an emulsion, similarly prepared but containing cresol, in which the drop size was 4 microns. At the same oil strength (1 per cent by volume), the second emulsion was twice as toxic as the first.

The results from the experiments with gas oil show even more distinctly the effect of drop size on toxicity. The toxicity of the emulsion with large oil droplets (7 to 8 microns) is approximately two times greater than that of the emulsion containing droplets of small size (2 microns), when the same concentrations are compared.

The emulsions containing the more volatile oils (kerosene and gas oil) were, as has generally been observed, less toxic than the mixtures

made with oils of the lubricating type, requiring two or more times the concentration of oil to produce the same effect.

The experiments with emulsions containing liquid petrolatum indicate that when the drop size is 2 microns or less, 95 per cent or more of the aphids are killed only when the oil concentration is raised to about three times that required of emulsions containing large oil droplets (8 to 10 microns). The experiments with kerosene and gas-oil emulsions show a similar relation.

RELATION OF DROP SIZE TO RETENTION OF OIL BY SPRAYED PLANTS

Emulsions in which the oil droplets were large spread readily over the foliage and stems, and, upon evaporation of the aqueous portion, left a nearly continuous coating of oil on them. This film of oil was indicated by the dark color of the sprayed foliage. Emulsions in which the oil droplets were relatively small spread poorly. The liquid collected in drops on the sprayed foliage and stems, and, upon evaporation of the water, left no continuous coating of oil, but only scattered oily spots.

Efforts were made to show this relation quantitatively. Plants were sprayed with diluted emulsions of known oil concentration. The emulsion that dripped from the plant during the spraying operation was collected, and the quantity of oil remaining on the plant was determined. The oil concentrations in the original dilute emulsion and that collected from the plants were determined by the modified Babcock method (*4*). Approximately the same quantity of nasturtium and bean foliage and of apple and peach twigs (without leaves) was used in each test. Nearly the same quantity of dilute emulsion was applied at a pressure of 5 pounds. The results shown in Table 3 are estimates only of the quantity of oil retained by the plants under these conditions.

TABLE 3.—*Quantity of oil retained by sprayed plants*

Preparation No.	Plants	Oil	Emulsifier	Method of emulsification	Size of oil drops	Concentration of oil by volume		Oil remaining on plants	Mean values (based on last column)
						Before application	In drip from plants		
118	Nasturtium plants	Paraffin oil (91 seconds viscosity).	Potassium fish-oil soap	Hot, 40-pound pressure	*Microns* 10	*Per cent* 0.95	*Per cent* 0.6	*Per cent* 36.8	*Per cent*
137	do	Naphthene oil 202	do	do					
137	do	do	do	do	8–10	2.02	1.73	14.3	
121	do	Paraffin oil (91 seconds viscosity).	Potassium fish-oil soap and cresol.	Cold-stir method	8–10	2.02	1.73	14.3	21.8
143	do	Naphthene oil 202	do	do	2	1.00	.90	10.0	
143	do	do	do	do					
118	Bean plants	Paraffin oil (91 seconds viscosity).	Potassium fish-oil soap	Hot, 40-pound pressure	2	1.92	1.92	0	
118	do	do	do	do	2	1.92	1.92	0	3.3
137	do	Naphthene oil 202	do	do	10	.95	.60	36.8	
137	do	do	do	do	10	2.00	1.40	30.0	
137	do	do	do	do	8–10	2.02	1.15	43.1	
121	do	Paraffin oil (91 seconds viscosity).	Potassium fish-oil soap and cresol.	Cold-stir method	8–10	2.02	1.15	43.1	
121	do	do	do	do	8–10	2.02	.96	52.5	41.1
143	do	Naphthene oil 202	do	do	2	1.00	.90	10.0	
143	do	do	do	do	2	2.00	1.80	10.0	
137	Peach twigs	do	Potassium fish-oil soap	Hot, 40-pound pressure	2	1.92	2.02	0	
137	do	do	do	do	2	1.92	2.02	0	5.0
143	do	do	Potassium fish-oil soap and cresol.	Cold-stir method	8–10	2.02	1.54	23.8	
143	do	do	do	do	8–10	2.02	1.63	19.3	21.3
118	Apple twigs	Paraffin oil (91 seconds viscosity).	Potassium fish-oil soap	Hot, 40-pound pressure	2	1.87	1.92	0	
118	do	do	do	do	2	1.87	1.92	0	0
121	do	do	Potassium fish-oil soap and cresol.	Cold-stir method	10	1.73	.91	47.4	
121	do	do	do	do	10	1.73	.67	61.3	54.3
					2	1.92	1.92	0	
					2	1.92	1.68	12.5	6.2

In every case, more oil remained on the plant after spraying with emulsions having large oil droplets than after spraying with emulsions in which the droplets were small. The higher absorption results obtained with bean foliage and apple twigs appear to be due to the ability of the emulsions to wet these plant surfaces more completely than those of the nasturtium foliage and peach twigs. This difference is possibly due to the different types of plant surface involved, but more extensive and careful studies would be necessary to substantiate such a claim.

SUMMARY AND CONCLUSIONS

Petroleum-oil emulsions were prepared (1) while hot, under pressure, (2) by a cold-stir method, and (3) by grinding in a colloid mill, to give a series of preparations, with or without emulsifiers, in some of which the oil droplets were large (7 to 12 microns in diameter) and in others small (2 to 4 microns or less in diameter).

The emulsions in which the oil droplets were relatively large were decidedly more toxic to *Aphis rumicis* than those in which the droplets were small.

The toxicity of the preparations, as correlated with drop size, was not influenced by the physical characteristics of the oil, the presence or absence of a soap emulsifier, or the presence or absence of cresol.

When foliage or twigs of plants without foliage are sprayed with emulsions of large drop size more oil is retained by the plant surface than when the foliage or twigs are sprayed with emulsions of small drop size. The following explanation for this is offered:

Oil droplets in emulsions and plant surfaces bear negative electrical charges. The droplets in an emulsion of small drop size have a greater charge per unit volume of oil than those in an emulsion of large drop size because the charge is proportional to the surface. It is believed that plant surfaces repel the droplets in the first type of emulsion with a greater force than they repel the droplets in the emulsions of large drop size, and that consequently the electric charges of plant surfaces and oil droplets are a factor in determining the ability of the oil in an emulsion to adhere to plant surfaces.

Under conditions of comparable concentration and type of oil, miscible oils are probably less toxic to insects than the ordinary soap-oil emulsions, because they contain smaller oil droplets and the oil therefore adheres to the plant (and no doubt to the insect) less effectively.

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THREE NEW HYMENOPTEROUS PARASITES OF THE PINE TIP MOTH, RHYACIONIA FRUSTRANA (COMSTOCK)[1]

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The following new Ichneumonidae and Braconidae were reared by the writer and others during a preliminary study of the parasites of the pine tip moth in connection with a project to introduce the parasites of this species into the Nebraska National Forest for the control of the tip moth there.

Campoplex frustranae, new species.

Campoplex frustranae may possibly be one of the many species recently described by Viereck;[2] but in his key to species it runs nowhere convincingly. It corresponds most closely to *angularis* Viereck, but differs in its pale scape and pedicel and in other characters.

Female.—Length 5 mm., antennae 3 mm., ovipositor 1.5 mm. Temples convexly sloping; diameter of lateral ocellus subequal to ocellocular line and slightly more than half postocellar line; eyes shallowly concave opposite antennae, frons only slightly broader than face; combined face and clypeus almost exactly as broad as long; clypeus broadly rounded at apex, medially very slightly produced; malar space about two-thirds basal width of mandible; antennae stout, flagellum thicker in middle than at either end, first joint four times as long as thick, others gradually shorter, those beyond apical fourth as thick as long and submoniliform. Thorax of normal proportions, propodeum with strong carinae, finely granularly opaque except apical areas, which are coarsely transversely rugose, areolar and petiolar area confluent, latter barely concave; areolet small, petiolate, radius beyond middle; nervellus inclivous, broken, but no trace of subdiscoidella present; legs moderately stout; hind basitarsus slightly longer than next two combined. Abdomen rather slender, weakly compressed; first tergite slightly longer than second, this half as long again as third and distinctly longer than broad at apex, spiracle slightly beyond middle; ovipositor sheath more than half as long as abdomen.

Black; scape and pedicel below, mandibles, palpi, front and middle trochanters, and apices of their coxae stramineous; tegulae yellow; front and middle legs otherwise testaceous, their tarsi paler; hind coxae black, reddish at apex, femur ferruginous, tibia brownish stramineous with apex blackish, tarsus fuscous, paler at base; wings hyaline; abdomen immaculate black except slightly reddish apical corners of second tergite.

Male.—Except sexually essentially like female; second tergite more extensively reddish apically.

Type locality.—Falls Church, Virginia.

Host.—*Rhyacionia frustrana* (Comstock).

Type.—Cat. No. 40102, U.S.N.M.

Ten females and 10 males selected from a large series reared by the writer during July, 1924, and April, 1925.

The delicate white cocoon is spun inside the shattered pupal shell of the host.

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[1] Received for publication Jan. 5, 1927; issued June, 1927.

[2] VIERECK, H. L. A PRELIMINARY REVISION OF THE CAMPOPLEGINAE IN THE CANADIAN NATIONAL COLLECTION, OTTAWA. Canad. Ent. 57: 225, 1925; 58: 130, 143-149, 1926.

Phanerotoma rhyacioniae, new species.

This species is remarkable chiefly for its very strongly depressed abdomen.

Female.—Length 2.7 mm. Head slightly broader than thorax, granularly opaque, sides of frons and upper part of face more or less rugose; eye large, bulging, broadly oval, sparsely hairy, its longest diameter nearly twice the length of ocellocular line; malar space no greater than basal width of mandible; clypeus minutely bidentate at apex; antennae opposite upper fourth of eyes, scape hardly twice as long as thick, basal joint of flagellum about two and a half times as long as thick, joints toward apex changing gradually from cylindrical to nearly round moniliform. Thorax depressed, granularly opaque, notauli irregularly rugose, propodeum medially and apically coarsely irregularly reticulate rugose, laterally more finely so; second abscissa of radius hardly as long as first and distinctly shorter than second intercubitus; first and second abscissae of cubitus not quite continuous, the second cubital cell not pointed at base; nervulus postfurcal by barely its length. Abdomen very flat, the edges especially at apex only slightly rolled downward, apically narrowly truncate but not at all emarginate, finely granularly opaque, longitudinally rather weakly and sparsely striate except in basal middle of first tergite and on apical half of third; first and third tergites subequal in length, second shorter by about a fourth; ovipositor straight, slightly exserted.

Ferruginous; stemmaticum, apices of antennae, propodeum, and third tergite brownish; abdomen otherwise yellowish; coxae, trochanters, and front and middle femora stramineous, hind femur darker; hind tibia reddish below, with apical and subbasal blackish spots above, extreme base and space between spots whitish, the same pattern but less distinct on middle tibia, front tibia mostly dark, tarsi paler than tibiae.

Male.—Like female but eyes larger with malar space and ocellocular space somewhat shorter; subbasal joints of antennae about a half longer than thick.

Type locality.—Bogalusa, Louisiana.

Host.—*Rhyacionia frustrana* (Comstock).

Type.—Cat. No. 40103, U.S.N.M.

Described from four females and five males reared from infested pine tips collected by R. A. St. George in April, 1925. Some of the specimens are less contrastingly colored than the type.

Microbracon gemmaecola, new species.

In Muesebeck's key to the North American species of Microbracon [3] this species runs to couplet 65, where it agrees fairly well with the characters assigned to *argutator* (Say); but a comparison of specimens shows the antennae to be somewhat longer (very nearly as long as body in *gemmaecola*), with the subapical joints relatively longer. The head is somewhat thinner anteroposteriorly, and the hind tibia, except at the extreme base, and the tarsus are black.

Female.—Length 2.5 mm. Head hardly two-thirds as thick as broad, temples sloping; face, frons, and vertex laterad of ocelli finely shagreened, vertex behind ocelli and temples polished; malar space about three-fourths as long as first flagellar joint; space between eye and clypeal opening more than half as wide as opening; antennae 27 to 30 jointed (28 in holotype), very nearly as long as body, flagellum tapering slightly beyond middle, basal joints two-thirds as thick as long, subapical joints distinctly though not conspicuously longer than thick. Thorax smooth and polished; notauli with sparse hairs; mesoscutum posteriorly with scattered, coarse, shallow punctures; propodeum posteriorly faintly finely reticulated and with a stump of the median carina and radiating ridges; stigma slightly more than a third as broad as long, radius before middle, second abscissa of radius barely twice as long as first and more than half as long as third, with which it forms a slight angle, first intercubitus two-thirds as long as second abscissa of radius. Abdomen broadly oval, opaque, granularly punctate, second tergite as long as first and longer than third, more coarsely sculptured with an

[3] MUESEBECK, C. F. W. A REVISION OF THE PARASITIC WASPS OF THE GENUS MICROBRACON OCCURRING IN AMERICA NORTH OF MEXICO. U. S. Natl. Mus. Proc., v. 67, art. 8, 85 p., illus. 1925.

irregularly rugose area in basal middle, and medially emarginate at apex; ovipositor sheath barely as long as abdomen exclusive of first tergite, rather stout.

Pale yellowish ferruginous, with back of head, lateral lobes of mesoscutum, mesosternum, and propodeum stained with piceous; antennae, stemmaticum, tarsi, hind tibia except base, and apices of other tibiae black; wings hyaline, faintly infumate in basal half.

Male.—Runs to *argutator* in Muesebeck's key. Like female, but malar space much shorter; antennae longer and more slender, the subapical joints nearly twice as long as thick; second tergite less distinctly emarginate.

Type locality.—Nantucket, Massachusetts.

Host.—*Rhyacionia frustrana* (Comstock).

Type.—Cat. No. 40104, U.S.N.M.

Described from seven females and eight males reared during March, April, and May, 1926, by L. W. Bailey from host in pine tips at the type locality and two females and one male reared by the author from the same host at Falls Church, Va., May 17 to 21, 1925.

The individuals of the Nantucket series display comparatively little variation and that mostly in the extent of the piceous staining of head and thorax. The Virginia specimens lack this staining entirely.

EURYTOMA PARVA (GIRAULT) PHILLIPS AND ITS BIOLOGY AS A PARASITE OF THE WHEAT JOINTWORM, HARMOLITA TRITICI (FITCH) [1]

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INTRODUCTION

The life history of *Eurytoma parva* (Girault) Phillips [3] indicates strongly that it is very gradually breaking away from the habit of parasitism upon animals and is becoming phytophagous. As pointed out by Gahan (*1*),[4] this is perhaps a recent specialization. There are strong indications that some individuals may even now develop upon plant tissue alone. However interesting this insect may be to the biologist, it is of no less interest to the layman, since at present it is one of the most important parasites of a very destructive pest of wheat, *Harmolita tritici* (Fitch).

In the light of recent observations it seems almost certain that at least the majority of the American species of Eurytoma found associated with various species of Harmolita have a life history parallel with that of *Eurytoma parva*. This assertion is not founded upon actual observation but upon analogy. For example, *E. parva* begins to emerge at about the time that the peak of emergence of its host is reached. This time of emergence for a parasite is unusual, and yet it is true of all species of Eurytoma so far found associated with Harmolita. On the other hand, all of the other primary parasites of the jointworms thus far observed emerge about a month later than their hosts. Secondary parasites may emerge at any time after the temperature has become sufficiently high for them to develop. Thus far there is no evidence that Eurytoma may be a secondary parasite on the jointworms.

The writer (*6*) figured the adult of *Eurytoma parva* and briefly mentioned this very unusual feeding habit in Farmers' Bulletin 1006, of the United States Department of Agriculture, published in 1918, giving the insect Girault's manuscript name, *parva*. Unfortunately, Girault's paper was delayed in publication, thus making the writer responsible for the species.

In the present paper, not only are the facts relative to the biology of *Eurytoma parva* recorded in greater detail, but some of the structural characters of the larva are described and compared with those of the host larva.

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[1] Received for publication Feb. 8, 1927; issued June, 1927. A thesis presented to the academic faculty of the University of Virginia in candidacy for the degree of doctor of philosophy.

[2] The writer offers grateful acknowledgement to W. R. Walton and J. S. Wade, of the Bureau of Entomology, for the use of literature, and particularly for securing a translation of an important Russian article; to Mrs. W. J. Phillips and George T. Hamner for assisting in the breeding work; to W. A. Kepner, S. A. Rohwer, and A. B. Gahan for helpful criticisms of the manuscript; and to Adam G. Böving for helpful criticisms of the drawings and descriptions of the larvae.

[3] Order Hymenoptera, family Eurytomidae.

[4] Reference is made by number (italic) to "Literature cited," p. 758.

ORIGIN AND HISTORY

It is very difficult, if not well-nigh impossible, to determine definitely whether or not *Eurytoma parva* is a native American species, although perhaps the weight of evidence favors the negative.

The writer first began to study the habits of the jointworms in 1904. Infested cereal and forage plants were collected for a number of years over the greater part of the United States by various persons connected with the cereal and forage insect investigations of the Bureau of Entomology. During this time a great quantity of material was gathered, and many emerged adults were preserved for study. A number of species of Eurytoma associated with species of Harmolita from wild grasses were reared, but no record was found of the rearing of *E. parva* prior to 1913. Furthermore, there is no record in the bureau files of any species of Eurytoma having been reared from *Harmolita tritici* (Fitch), *H. vaginicola* (Doane), *H. grandis* (Riley), *H. secalis* (Fitch), *H. websteri* (Howard), or *H. hordei* (Harris) —these being the jointworms infesting the cereal crops—and this despite the fact that prior to 1913 jointworms from grains and grasses of eastern, central, and midwestern territory were being reared intensively. However, *E. pater* Girault was reared from jointworm-infested wheat stubble collected by the writer near Youngstown, Ohio, in 1911, according to bureau records. This is the first species of Eurytoma found associated with Harmolita from the small grains in America.

The first record of the occurrence of *Eurytoma parva* is dated June 4, 1913. The writer was then at Purdue University, La Fayette, Ind., where he was breeding various species of jointworms. While sweeping a field of wheat for *Harmolita grandis*, the writer captured a female of some species of Eurytoma. She was placed in a breeding cage on wheat that had been previously infested with *H. tritici*, and late in the same afternoon was observed to oviposit in the wheat stems. The following statement is made in the writer's notes of June 4, 1913: "I have never (to my knowledge) reared this from *H. tritici* and it will be an additional parasite for *tritici* should I succeed in rearing it." Four males were reared from this cage and have recently been identified by A. B. Gahan, of the Bureau of Entomology, as *Eurytoma parva*.

Although *Eurytoma parva* was first found in the Central States in 1913, in 1914 and 1915 it was much more plentiful in the Eastern States. Apparently it has spread gradually westward, as is well illustrated in Missouri. No specimens of *parva* had been reared from Missouri previous to 1917, so the writer and his associates introduced the species into one or two wheat fields of that State in 1918, 1919, and 1920. In 1920, however, *E. parva* was reared sparingly from collections made at localities in Missouri where the species had not been liberated. As a small number were introduced into that State at one or two points only, it seems safe to assume that at that time *E. parva* was just spreading into Missouri, independently of the introductions.

It seems strange that *Eurytoma parva* should not have been found in the collections made before 1913, since extensive collections and rearings of jointworms from the small grains had been made for nine years previous to that date. These rearings were from collections

made in practically every State where *Harmolita tritici* was known to be present. Moreover, the jointworms occurring in the small grains have been reared in America for many years, a fact which leads the writer to think that this parasite is possibly an introduced species.

The writer has made strenuous efforts to obtain from Europe named species of the genus Harmolita and some of their parasites, for comparison with American forms, but has been unsuccessful. However, Rimsky-Korsakov's (*8, p. 36–40*) descriptions and illustrations of adults and larvae leave little doubt that his (*Isosoma*) *Harmolita inquilinum* is a species of Eurytoma. Furthermore, its habits are similar to those of *E. parva*. It is singular that Hedicke (*2*), in his extensive work on the jointworms of Europe, fails to mention Rimsky-Korsakov's recently described species, or the latter's paper in which he records the parasitic and phytophagous habits of (*Isosoma*) *H. inquilinum*. Hedicke records only one American species, *H. hordei*.

CLASSIFICATION OF THE LARVAE OF EURYTOMA PARVA AND HARMOLITA TRITICI

The most recent and comprehensive work on chalcidoid larvae is by H. L. Parker (*4*). In this paper Parker divides the larvae into seven main groups, based largely upon the external structural characters of the first instar. *Eurytoma parva* apparently more nearly fits into Group VI, there being some few points of disagreement. Parker evidently made a slight error in drawing up his description of Group VI, for on page 333 of his work he states that the thoracic segments bear three pairs of long sensory hairs and that the abdominal segments bear four pairs, while on page 284 he states that the thoracic segments bear six and the abdominal bear four sensory hairs, which agrees with the figures. The first instar of *E. parva* has the same number of sensory hairs on both the thoracic and abdominal segments. There is a very indistinct band of most minute spicules on the body segments, and there appear to be five pairs of open spiracles, though the second thoracic spiracle is very small.

The larva of *Harmolita tritici* does not seem to fit into any of Parker's groups. The first-instar larva comes nearer to Group II, perhaps, than any other, though it has many points of difference from all the groups. For example, the head is not broader than the body, and is very feebly chitinized; there are apparently no antennae or sensoria except in the buccal region; apparently no open spiracles; no bands of spicules around the segments; and the mandibles are simple and triangular. Therefore the larva of *H. tritici* might well constitute another group, making a total of eight groups according to Parker's classification, the new one having the following characters: Cylindrical in form, tapering toward each extremity; 13 fairly distinct segments, not including the head; head about the size of the thorax, conical in outline, the mouth being located decidedly subterminally near the midline of the ventral surface; apparently no antennae, spicules, sensory hairs, or open spiracles; mandibles simple and triangular in outline.

In locating the body setae or sensory hairs of both *Eurytoma parva* and *Harmolita tritici*, the writer has decided to mention and locate only those for one-half of the body throughout its entire length. It will perhaps be less confusing to locate all the setae on

the head, and this has been done, though all lateral views of the larvae show only half the total number of setae. This should be borne in mind in reading the descriptions.

DESCRIPTION

HARMOLITA TRITICI (FITCH)

First Instar

Cylindrical in form, tapering toward each extremity; 13 fairly distinct segments in addition to the head; head about size of thorax, conical in outline, the mouth located decidedly subterminally near midline of ventral surface; apparently no antennae, spicules, sensory hairs, or open spiracles; two pairs of sensoria in maxillary region; mandibles simple and triangular in outline, whitish, and very feebly chitinized. Entire larva a translucent whitish color and very soft (fig. 1, B).

Average length of three individuals 0.74758 mm., average width 0.128565 mm. These measurements were taken from living specimens.

Full-Grown Larva

Cylindrical in form, tapering slightly toward each extremity, and having 13 segments, not including the head, which is almost hemispherical (fig. 1, A). Mouth parts situated approximately in middle of face or front of head. Head narrower than body segments. Antennae broader than long and scarcely visible without high magnification. Eight small sensory setae on the head, all located laterally except one pair, which is situated at the base of the labrum (fig. 1, A; fig. 2, A). The two most dorsal setae are about twice the length of any of the others. Labrum rather large and convex, with three fairly distinct lobes, the central lobe being the largest. Eight minute sensoria on labrum, a group of three at each side of mouth, and a pair on central lobe of labrum.

The ventral mouth parts form the entire lower part of the head; they are very convex, slightly lobed at the mouth, and bear 14 minute sensoria, 7 to each lateral lobe.

Mandibles simple, triangular, rather heavily chitinized, and brownish.

Thoracic segments somewhat longer than the abdominal; first thoracic distinctly the longest segment of the body, and bearing five very inconspicuous sensory hairs, there being one upper and one lower tergal, one lateral, one sublateral, and one sternal; second and third segments each bearing four sensory hairs, the upper tergal hair being absent; each of the remaining body segments bearing two sensory hairs, one tergal and one lateral, except the anal segment, which bears three. All sensory setae of the body very small and inconspicuous and arranged as indicated in Figure 1, A.

Average length of eight individuals 3.8942 mm., average width 0.8942 mm. The measurements averaged were taken from larvae fixed in Carnoy's fluid.

EURYTOMA PARVA (GIRAULT) PHILLIPS

Egg

The egg is ovoid with a long pedicel at one pole and a short pointed pedicel at the other (fig. 1, G). The length of the long pedicel is about one and a half times that of the egg. Eggs freshly dissected from the abdomen of a female are white; after oviposition they are of a dirty brownish color.

The average length of three eggs, including the pedicels, was found to be 1.0378 mm., and the average width 0.1515 mm. The measurements averaged were taken from specimens preserved in Stromsten's fluid.

First Instar

According to Parker's description, the shape of the first-instar larva is typical of the genus Eurytoma (fig. 1, E, F). Larva translucent, whitish in color, its segmentation very distinct; head brownish; head and body bearing numerous sensory hairs of different lengths.

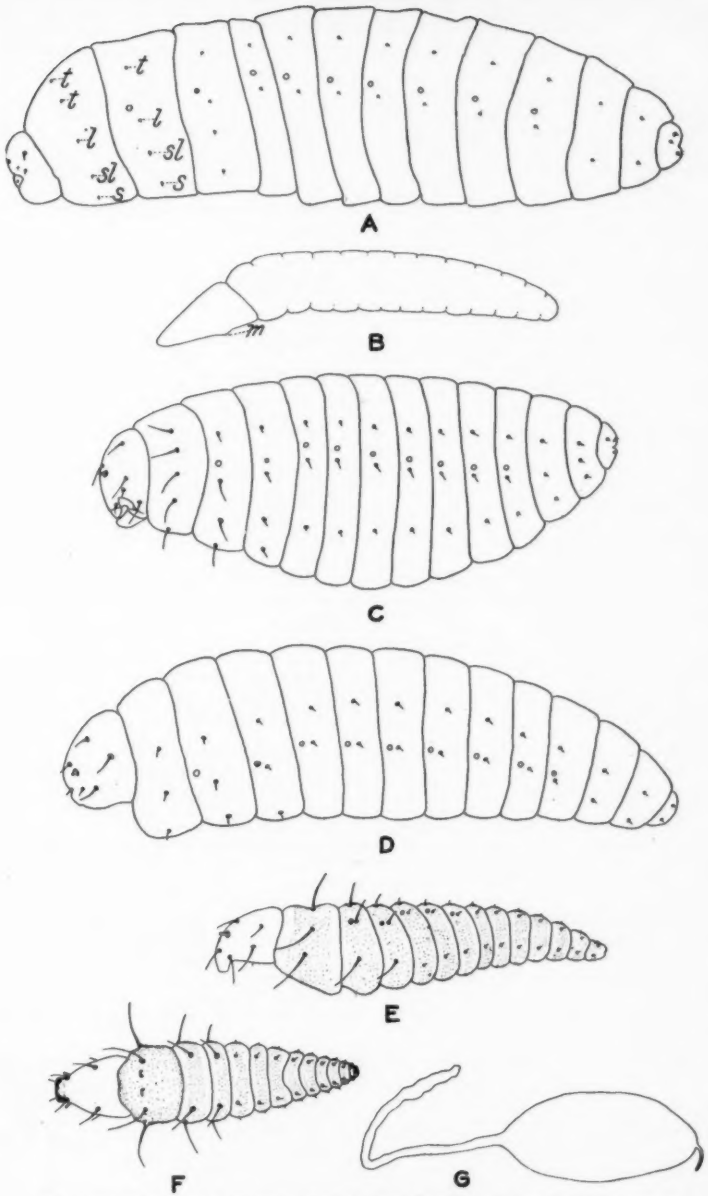

FIG. 1.—A, lateral view of full-grown larva of *Harmolita tritici*, showing position of sensory hairs: *t*, Tergal sensory hair; *l*, lateral sensory hair; *sl*, sublateral sensory hair; *s*, sternal sensory hair. B, lateral view of first-instar larva of *H. tritici*; *m*, mouth. C, lateral view of full-grown larva of *Eurytoma parva*, showing position of sensory hairs. D, lateral view of second-instar larva of *E. parva*, showing position of sensory hairs. E, latero-ventral view of first-instar larva of *E. parva*, showing position of sensory hairs. F, ventral view of first-instar larva of *E. parva*, showing chitinized plate of first thoracic segment, somewhat dorsally flexed. G, egg of *E. parva*. A and C are drawn to the same scale; B, D, E, F, and G are drawn to a uniform scale, but are more highly magnified than A and C

Average length of five living larvae 0.7880 mm., average width 0.1884 mm.

Outline of head (fig. 1, E, F) reminds one somewhat of the beak of a vulture, approximately as wide as thick, but distinctly longer than wide, brownish in color, and strongly chitinized; two antennae, approximately three times as long as broad, but with only one segment; head bearing 10 prominent sensory hairs, of which 4 are on the ventral surface, a pair just above the labrum, a pair just above the antennae, and 1 hair on each lateral face (fig. 1, E). Mouth situated on ventral side, at apex of the somewhat elbow-shaped head, and surrounded by a heavily chitinized ring; mandibles sickle-shaped, strongly chitinized and almost black, the bases brownish; labrum with six very indistinct sensory organs; sensory organs of labium so minute that the writer could not identify them with certainty. Parker (*4*) states that *Eurytoma dentata* Mayr has a pair of sensory organs below the mouth, but does not place them. He does not mention any sensory organs for *Eurytoma rosae* Nees.

Body composed of 13 segments, the thoracic segments being the longest, and the remaining segments tapering sharply posteriorly. Each thoracic and each abdominal segment bearing three sensory hairs; those of the first thoracic segment long and coarse, those on the remaining segments gradually diminishing in size and length until those of the last two or three segments are almost invisible. These sensory hairs are arranged in three rows (a lateral row near the spiracles, a tergal row, and a sternal row), each extending the full length of the body. There is more or less of a band of very minute spicules around each segment, visible only with high magnification. Five spiracles, all apparently open.

Ventral surface of first thoracic segment shaped somewhat like the sole of a square-toed shoe, strongly chitinized, and having a pair of chitinous processes near center (fig. 1, F).

When the larva is ready to molt, the head is much smaller than the thoracic segments, all the body segments being much more robust.

Second Instar

In the first molt the larva undergoes a great change in appearance. The second instar resembles neither the first instar nor the full-grown larva, though it is more like the latter than the former. The larvae appear to be more delicate while in this instar than at any other time.

Head somewhat intermediate in shape between that of first instar and that of full-grown larva (fig. 1, D). Head capsule very feebly chitinized; first thoracic segment extending far below the ventral surface of the head; head whitish and bearing 12 sensory hairs, of which 8 are large and 4 small; 4 of the large hairs are on the cephalic aspect of the head, just above the antennae, 1 is on each lateral face, and 1 laterad of each mandible; of the small sensory hairs 2 are just above the labrum, and 1 laterad of the base of each mandible; antennae about twice as long as wide; mouth located on apex of head; mandibles triangular in outline, very feebly chitinized, and bearing a single denticle; sensory organs of labium appearing so inconspicuous and indistinct that the writer can not enumerate them with accuracy; each thoracic segment bears 3 sensory hairs and the abdominal segments 2 each, arranged as indicated in Figure 1, D; all sensory hairs except those on the last two or three abdominal segments much smaller than those in any other instar. Nine pairs of open spiracles.

Average length of six individuals (living specimens) 1.4147 mm., average width 0.4096 mm.

The writer has definitely identified as many as three instars, the first, the second, and the full-grown larva. After the second instar the larvae so closely resemble the full-grown larva that it has been impossible to identify the third and fourth with certainty. There are, however, apparently four molts, making five instars.

Full-Grown Larva

Full-grown larva somewhat barrel-shaped, distinctly broader in midregion and tapering rather sharply toward each extremity. Body composed of 13 segments, not including the head; head almost hemispherical, narrower than the body segments, and translucent-whitish except in mandibular region, where it is brownish; mouth parts pointing downward; antennae distinctly longer than broad; on the head 12 sensory setae, 8 large and 4 small, the latter situated just above bases of mandibles; of the large setae 4 are on the dorso-cephalic

aspect of the head, above the antennae, one on each side of head, near center of lateral face, and one caudad of base of each mandible (fig. 1, C, and fig. 2, D). Labrum three-lobed and bearing eight minute sensoria, there being a group of three near each corner of mouth and a pair on central lobe; the ventral mouth parts form, together, a fused region, which anteriorly is trilobed, corresponding to the component median labial, and the two lateral maxillary regions. This fused region (fig. 2, D) bears 16 sensory hairs and organs, 8 on each lateral lobe or maxillary region; each lateral lobe bears one comparatively large circular structure, which is possibly a vestigial maxillary palp; it is rather strongly chitinized, convex, and extends above the surrounding surface of the lobe or maxillary region; each maxillary region has 4 rather prominent sensory hairs, or setae, and 3 inconspicuous sensory hairs, or papillae. (See fig. 2, D.) In the legends to the illustrations these have been called setae and papillae, respectively. The mandibles (fig. 2, G, H) are heavily chitinized, almost black, triangular in outline, and each has one large denticle on the inner margin. The position of the mandibles and their articulation to the different parts of the mouth frame are shown in Figure 2, F. For an interpretation of these parts the legend of Figure 2 may be consulted. The thoracic segments gradually increase in size posteriorly; the first thoracic segment is the longest and the third abdominal segment the thickest body segment. The first thoracic segment bears 5 prominent sensory hairs, the second and third 4 each, and the abdominal segments 3 each; these abdominal sensory hairs are arranged laterally in three longitudinal rows (fig. 1, C). The sensory hairs of the first and second thoracic segments are conspicuously longer and larger than those of the remaining segments of the body. There are nine open spiracles.

Average length of 100 living specimens 2.7330 mm., average width 1.1752 mm.

SEASONAL HISTORY

In the vicinity of Charlottesville and Warrenton, Va., the adults of *Eurytoma parva* emerge from about the beginning to the middle of May, the time depending upon weather conditions. Apparently there is a preoviposition period of several days, though, since the species does not breed very freely in confinement, this point is rather difficult to determine.

Adults lived in confinement for nearly a month, a much longer time than jointworm adults live under similar circumstances. This comparatively long life, if we assume that it normally obtains under field conditions, greatly increases the opportunity of the females to find suitable places for oviposition.

The eggs hatch in about four or five days, the larva rupturing the eggshell at the base of the long pedicel. The larva apparently passes through four molts, though this fact could not be definitely determined, and becomes full grown in about 24 days. It then remains in the larval stage in its cell in the wheat stubble until the following spring. Excrement is voided in the cell after the larva becomes full grown, and usually just before the prepupal stage. This unusual habit has been noted by the writer and Poos (*7, p. 413, 419, 425*) in a previous paper on the jointworm parasites, and by others in the case of Donacia, Myrmeleo, and the rice weevil. In the three cases last named the excrement when voided is used in building up the cocoon and is apparently reserved for this purpose. In the case of *Eurytoma parva* and other jointworm parasites no cocoon is formed. The food of these small Hymenoptera perhaps contains very little matter that is not entirely digested and absorbed, and hence no fecal matter is elaborated. Besides, if excrement should be voided at regular intervals, their food supply might be contaminated, and this perhaps might lead to complications arising out of bacterial growth. Some time in the latter half of March the larvae change to pupae, and the adults emerge in the first half of May.

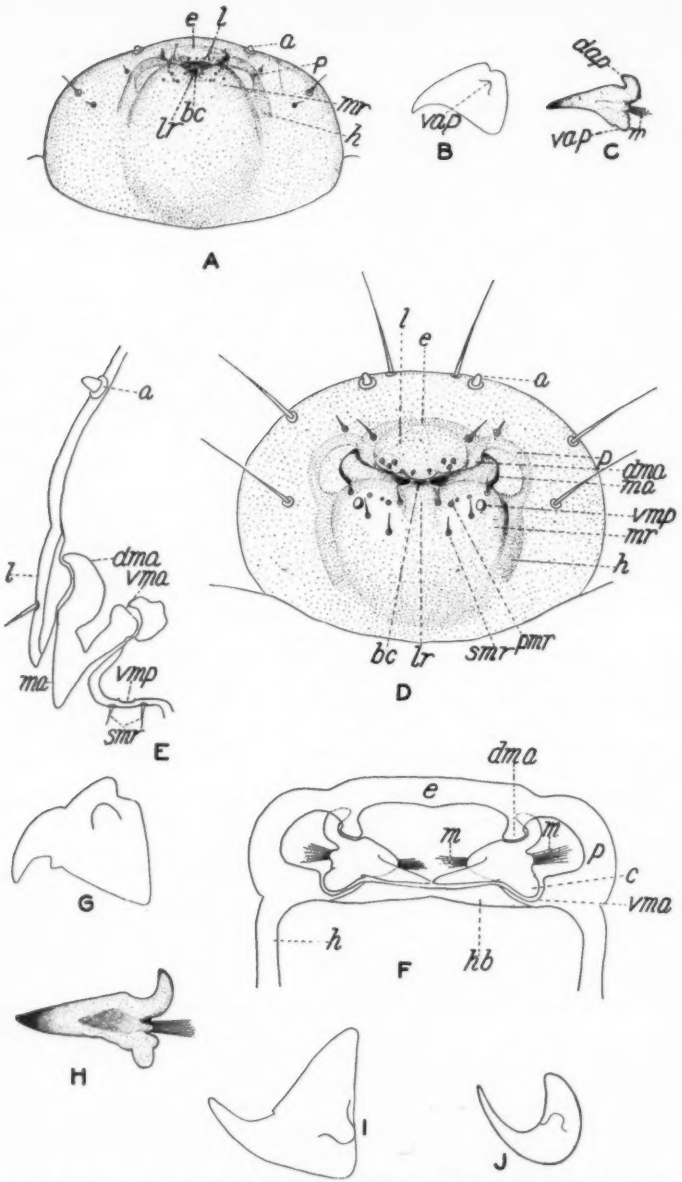

FIG. 2.—A, ventral view of head of full-grown larva of *Harmolita tritici*, showing position of mouth parts; B, ventral view of mandible, showing condylar process; C, exterior-lateral view, showing dorsal and ventral articular processes; D, ventral view of head of full-grown larva of *Eurytoma parva*, showing position of mouth parts; E, longitudinal section through maxillary region, showing labrum, mandibular articulations, what has provisionally been termed the vestigial maxillary palp, and two adjacent sensory hairs of maxillary region (the cuticle in this region had shrunk in such a manner as to involve the "vestigial maxillary palp" and three sensory setae in the same plane with the antenna); F, mouth frame of full-grown larva of *E. parva;* G, ventral view of mandible of full-grown larva of *E. parva*, showing condylar or ventral articular process and denticle on inner margin of mandible; H, exterio-lateral view of same, showing dorsal and ventral (condyle) articular processes and muscular attachment; I, ventral view of mandible of second instar of *E. parva;* J, ventral view of mandible of first instar of *E. parva*. (For explanatory legend see p. 751.)

The records on individual farms at Warrenton, Va., since 1916 show that *Eurytoma parva* has gradually increased in numbers at the expense of *Harmolita tritici* until at present it is one of the most important parasites of the wheat jointworm. The bureau records are not so complete for other parts of the country, but the indications are that in the Central States the trend is in the same direction.

NATURAL ENEMIES

Eurytoma parva, like *Harmolita tritici*, suffers from the attacks of parasitic enemies, though not to the same extent. The lower susceptibility of the former to attack by enemies appears to be due to the fact that the full-grown larva is very active, whereas the larvae of *H. tritici* are very sluggish. The least disturbance will cause a larva of *E. parva* to wriggle vigorously. Undoubtedly by their activity these larvae crush many enemy larvae of the first instar that attempt to attack them. This has actually been observed in some instances.

Four parasites have been found attacking larvae of *Eurytoma parva*—*Digfopinotus aureoviridis* Crawford, *Eupelminus saltator* (Lind.), *Eupelmus allynii* (French), and *Homoporus chalcidiphagus* (Walsh and Riley). The last named would probably be only occasionally successful in its attacks; its larva is very sluggish and while in the first instar would very easily be crushed by the very active larva of *E. parva*.

BREEDING METHODS

The usual type of flowerpot breeding cage with a glass cylinder for a cover was used for the majority of observations in 1924, at Charlottesville, Va., and fairly good results were obtained; in fact, a greater degree of success was achieved than at any previous time, perhaps because of a special method of handling the adults. As they emerged they were transferred to glass cylinders about 1½ inches in diameter and about 10 inches long. One end of each cylinder was covered with cheesecloth and the other plugged loosely with absorbent cotton. A little melted beeswax was poured on the inside of the cylinder and smoothed out flat, and a droplet of 17 per cent (approximately) sugar solution was placed on the beeswax for the adults to feed on. The beeswax kept the sugar solution from spreading over the side of the glass cylinder, where it would entangle the parasites. These stock cages were placed in a cool, dark place; the adults could in this manner be kept alive for two or three weeks, as a reserve supply from which to replenish the breeding cages. Previously the writer had been unable to maintain a reserve of the adults until the plants infested with the jointworm were in proper condition for the parasites to oviposit.

EXPLANATORY LEGEND FOR FIG. 2

A and D are drawn to the same scale; B, C, E, F, G, and H are drawn to a common scale, but are more highly magnified than A and D; I and J are drawn to a common scale, but are much more highly magnified than the others.

Explanations of symbols: *a*, Antenna; *bc*, buccal cavity; *c*, condyle; *dap*, dorsal articular process of mandible; *dma*, dorsal mandibular articulation; *e*, epistoma; *h*, hypostoma; *hb*, hypopharyngeal bracon; *l*, labrum; *lr*, labial region; *m*, muscle; *ma*, mandible; *mr*, maxillary region; *p*, peristoma; *pmr*, papillae of the maxillary region; *smr*, setae of the maxillary region; *vap*, ventral articular process or condyle; *vma*, ventral mandibular articulation; *vmp*, "vestigial maxillary palp."

All who breed small Hymenoptera know that closely related forms are often difficult to recognize and separate while alive, particularly if there are a number of individuals in the same breeding jar. For different breeding experiments it often becomes necessary to separate these living forms with certainty—no small matter, if several species and many specimens of each are involved. Eight or ten years ago the writer hit upon a very workable plan which has admirably met this difficulty. It may be known to some workers, and of interest to others.

These small Hymenoptera are collected from the breeding jar and placed indiscriminately in small vials. The plugged end of the vial is turned downward for a few minutes, whereupon the insects collect in the upper end. The plug is then quickly removed, the mouth of the vial held over a small cake of ice, and the end of the vial is given two or three vigorous taps with the fingers. The insects fall on the cake of ice, and all are immediately benumbed. The ice is then placed in a shallow saucer in which the specimens may be examined under a microscope and the species separated. The ice used should be nearly flat on both the upper and lower surfaces, so that the insects will not slide off.

When the specimens are removed from the ice those of each species should be placed in a vial with strips of absorbent paper, and in a few minutes they will become dry and be as active and alert as ever, apparently none the worse for the experience. In this way the vials may be stocked with the required number of individuals of each sex. When they have become dry they are ready to be transferred to the breeding cages.

Besides the method here described, involving the usual flowerpot breeding cage, other methods were tried for the purpose of demonstrating particular features of the life history of *Eurytoma parva*. Three methods of rearing larvae of *E. parva* in cell slides were tried, all resulting in failure, though 16 slides were used in each experiment. The cell slides were made by grinding with a dentist's burr a cavity 5 mm. long, 2 mm. wide, and 1.5 mm. deep, in a piece of common glass about two-thirds the size of an ordinary glass slide. A first-instar larva of *E. parva* was dissected from a cell of *Harmolita tritici* and placed in a cell slide on a full-grown larve of *H. tritici*, and a cover glass was partly sealed down over the cell with a droplet of honey. Another method was simply to place the cover glass over the cell and then put the slide in a moist chamber. A third method was to place the cover partly over the cell and put the slide in a moist chamber. All of the slides were kept in darkness in a desk drawer.

Two methods involving transfer were tried, one a failure, the other but slightly successful. Suitable plants in breeding cages (pot cages) were selected and a cell of *Harmolita tritici* containing a full-grown larva was taken from one and carefully opened under a binocular microscope. A larva of the first or second instar of *Eurytoma parva* was carefully removed from its cell and placed on the full-grown larva of *H. tritici* in the cell just previously opened. The cell was then closed very carefully and wrapped with the end of a green leaf from the plant from which the larva of *H. tritici* was taken. It was hoped that the green leaf would prevent evaporation and make conditions more nearly normal.

By the second transfer method a tender wheat stem was located in one of the pot cages that was infested with *Harmolita tritici*, and a tender gall then located in which the host larva had but recently hatched. The cell was carefully opened with a fine needle, under a binocular microscope, the host larva removed, and in its place was inserted a first-instar larva of *Eurytoma parva* that had not previously fed on the larvae of *H. tritici*. The cell was then carefully closed and wrapped in a green leaf, as in the method described in the preceding paragraph.

Ten trials were made of the first transfer method, only one of which was successful. In this case a larva of the second instar was placed on a full-grown host larva. The parasite larva was removed from a cell in which it had evidently devoured a young host larva and had then begun to feed on the sap of the plant. This parasite larva completed its development upon the larva of *Harmolita tritici* and evidently was unable to secure plant sap, as the appearance of the plant tissue where the host larva was confined indicated that the tissue dried up soon after the transfer was made. The only striking thing about this parasite larva was the fact that it was slightly below normal in size.

Nine trials, all failures, were made of the second transfer method. The results, however, do not have the ordinary value of negative results, because in no case did the plant tissue appear to heal after the operation; in every instance it died and dried out. Plainly, there was absolutely no way for the young larvae to obtain food from the plant tissue.

The situation may be summed up as follows:

1. *Harmolita tritici* must oviposit in a wheat stem before a female *Eurytoma parva* will deposit eggs in the stem, although *E. parva* places a great many eggs outside the jointworm cells.

2. When an egg of *Eurytoma parva* hatches just outside a jointworm cell and there is no host larva in its immediate vicinity it proceeds at once to feed on the sap of the plant. The inference is, therefore, that it may reach maturity on plant sap alone.

3. Should a larva of *Eurytoma parva* find itself upon a mature larva of *Harmolita tritici* it is possible for the former to reach maturity by feeding upon this mature larva instead of upon plant tissue. Such cases would ordinarily be rare under field conditions, since larvae of *H. tritici* are usually in about the first or second instar when the larvae of *E. parva* hatch, and these small larvae do not supply food enough to carry the *E. parva* to maturity.

4. In the case of perhaps most frequent occurrence, the larva of *Eurytoma parva* while in its first instar feeds on the larva of *Harmolita tritici*, and then changes to a plant diet.

It will be seen that the larva of *Eurytoma parva* is at present in a highly plastic or adaptive condition, a fact which indicates to the writer that it is in the process of changing over from parasitism upon animals to phytophagy, while as yet entirely dependent upon *Harmolita tritici* for the stimulus leading to oviposition.

COMPARISON WITH THE HOST LARVA

The most striking differences between the larvae of *Harmolita tritici* and those of *Eurytoma parva* occur in the first instar (fig. 1, B, E, F). The larva of *H. tritici* is apparently a most delicate

organism, and capable of only slight movement. Its mandibles are extremely minute and feebly chitinized; setae, if present, are too small or inconspicuous to be seen under a magnification of 400 diameters; no open spiracles are apparent, the body segments are barely indicated, and all are thinly membranous. In contrast to this larva the first-instar larva of *E. parva* (fig. 1, E, F) is a rugged, motile organism. The mandibles are stout, large, highly chitinized, and dark brown in color; the setae are numerous, long, and stout; there are five pairs of open spiracles; the body segments are well defined, and the entire ventral surface of the first thoracic segment appears to be heavily chitinized.

As previously stated, the differences in the remaining instars are much less striking, but the larva of *Eurytoma parva* is much more active, and the setae and mouth parts more strongly developed. The shape of the larvae in the two species is characteristically different throughout. The full-grown larva of *E. parva* is very active, and that of *Harmolita tritici* sluggish; the two are quite different in shape (fig. 1, A, C); the mouth parts of *E. parva* are more prominent and more highly chitinized; the setae are more numerous and much larger and stouter; the color of both larvae is almost a canary yellow.

The larger and more highly chitinized mandibles serve the full-grown larva of *Eurytoma parva* to very good advantage. During its last instar the wheat stems are much more woody, and there does not appear to be such a generous flow of plant sap. This larva is therefore obliged to shred and lacerate the walls of its cell more vigorously in order to maintain a good flow of sap; this fact will account for the large amount of frass in the cells, as already mentioned by Rimsky-Korsakov (*8, p. 19–20, 36–40*) in the case of *Harmolita inquilinum*. This writer, however, apparently failed to interpret correctly the connection between the accumulated frass, the stronger mandibles, and the mode of life of the parasite. The larvae of *H. tritici* mature earlier, while the wheat plants are more succulent, the bodies of the larvae being almost constantly bathed in the rich plant sap. They need then to lacerate the plant tissue but slightly to maintain the flow of sap, and naturally less frass is accumulated.

This feature is more strongly emphasized in *Harmolita grandis* (Riley), *H. websteri* (Howard), and *H. albomaculata* (Ashm.). In fact, all species of Harmolita that develop within the center of the stem, where they are not surrounded by a plentiful flow of sap such as surrounds those which, like *H. tritici*, develop in the tissues of the stem walls, are forced to tear and lacerate more extensively the walls of the stem in order to maintain a constant supply of sap. As a result, a greater amount of frass is always collected under these conditions.

The writer has not had an opportunity to make a detailed study of the internal anatomy of either *Harmolita tritici* or *Eurytoma parva*, but from a casual examination of stained sections there appears to be a noticeable difference in the development of the salivary glands of the first and perhaps the second instars of the two species. These glands are noticeably larger, and the gland cells themselves are larger, in the vegetable feeder *H. tritici* than in *E. parva*, which, in these instars, is parasitic upon animal tissue. In the full-grown larvae, however, which in both species are vegetable feeders, the glands are more nearly of the same size.

INJURY TO THE PLANT

To speak of plant injury in discussing the biology of an insect parasite sounds a little odd, perhaps, but the question may properly be asked whether *Eurytoma parva* is a parasite or whether it should not be classed as a plant pest. The writer does not hesitate to class it as a parasite. The wheat plant is damaged perhaps more severely by the larvae of this insect when they are once established within it than by the larvae of *Harmolita tritici;* but the point is that the wheat plant would not be attacked by *E. parva* at all if larvae of *H. tritici* were not present in the stem walls. All larvae of *H. tritici* with which a larva of *E. parva* comes in contact are destroyed; the numbers of the host are therefore reduced and actual damage to wheat, on the part of *H. tritici,* is thus curtailed.

The larvae of *Eurytoma parva* deform the wheat stalks and deplete the nourishment in the same manner as do those of *Harmolita tritici,* and perhaps inflict slightly greater injury, since they feed a little longer and are obliged to shred the tissue more extensively in order to maintain a flow of sap. Externally, the conformation of the individual cells and the general aspect of the distorted wheat stem are so similar in the case of both insects as to render identification from these characters impossible. The cells of *E. parva* are often somewhat darker in color than those of *H. tritici,* but this character is not always reliable.

PARTHENOGENESIS

Two breeding cages were used in 1924 to test parthenogenesis on the part of *Eurytoma parva.* Only virgin females of *E. parva* were placed in the cages; they oviposited about as freely as those in the other cages, and the act of oviposition seemed normal. All the cells of the jointworm galls in these two cages were carefully opened April 9, 1925, and a few unemerged adults of *Harmolita tritici* were found, the remaining cells containing dead larvae which could not be identified. This evidence is inconclusive, though the fact that the virgin females appeared to oviposit normally and freely, and the further fact that nearly related species have proved to be parthenogenetic, make it seem probable that *E. parva* is also parthenogenetic.

PARASITIC AND PHYTOPHAGOUS HABITS OF THE LARVA

So far as the writer has been able to learn, only two cases of feeding habits such as those here described have been observed by other investigators on the Chalcidoidea. Nielsen (*3, p. 46*), in Denmark, made a very brief statement to the effect that he found an undetermined species of Eurytoma that first destroyed the young larvae of *Cryptocampus angustus* Htg., which lived in the heartwood of willow twigs, and afterwards fed and completed its development in the pith of the willow stems. Phillips simply mentioned this feeding habit for *E. pater* Girault in 1917 (*5, p. 145*), and for *E. parva* in 1918 (*6*). In 1914 Rimsky-Korsakov (*8, p. 19–20, 36–40*) gave an excellent account of similar habits of (*Isosoma*) *H. inquilinum* (Rimsky-Korsakov) in rye, although there are in this connection a number of interesting points that he was unable to clear up.

From Rimsky-Korsakov's description of the adult of (*Isosoma*) *Harmolita inquilinum*, and from the illustrations of the adults and the larva, the writer feels sure that American systematists would place it as a Eurytoma. The host, *H. rossicum* (Rimsky-Korsakov), develops in rye stems. The infested sections of rye stalks bearing the galls are illustrated in Figures 19 and 20, on pages 32 and 33, respectively, of the work cited. Some of these galls resemble very closely the galls formed by the American species *H. secalis* (Fitch), while the galls on other sections resemble very closely those of the American species *H. vaginicola* (Doane) on wheat. The illustrations of *H. rossicum* are typical of American *Harmolita*.

Ever since 1917, when it was learned that the larvae of Eurytoma are both parasitic and phytophagous, the writer has been deeply interested in the species of Eurytoma that are associated with Harmolita. Difficulty in securing identification of the adults, failure to breed them freely in confinement, and their apparently slight economic importance somewhat discouraged further investigations at that time. In recent years *E. parva* has taken first rank as a parasite of the wheat jointworm, systematists have become willing occasionally to risk identification of material, and a greater measure of success has been obtained in rearing specimens under observation.

The majority of the species of Harmolita observed will oviposit freely in confinement without taking food, and oviposition begins soon after emergence; while *Eurytoma parva* apparently has a much longer preoviposition period and requires food. By keeping the adults confined in vials plugged with cotton, supplying them with sirup containing 17 per cent of sugar before placing them in breeding cages, and feeding them while in those cages, a fair measure of success was obtained.

A number of females of *Eurytoma parva* were dissected, and in no case were more than two or three mature eggs found in an abdomen, even in newly emerged specimens dissected before they had an opportunity to oviposit. Adults of the wheat jointworm have from 50 to 100 eggs in the body cavity before oviposition begins.

Females of *Eurytoma parva* refuse to oviposit in stems in which *Harmolita tritici* has not previously deposited eggs. Furthermore, a large number of dissections of field and cage material indicate that the females of *E. parva* prefer to oviposit in cells of *H. tritici* containing a larva of the first or second instar, as it is very rare to find larvae of *E. parva* on larger larvae of *H. tritici*; eggs and young larvae of *E. parva* were practically always found upon larvae of the first or second instar, even though older larvae of *H. tritici* were present in the stem. This condition is easily brought about both in the field and in the breeding cages, since the adults of *H. tritici* emerge in advance of those of *E. parva*, and since the adults of *E. parva* live much longer. The latter have been kept alive for about a month, while a week or 10 days is about the limit of adults of *H. tritici*.

The female of *Eurytoma parva* may deposit from one to six eggs in the immediate vicinity of a larva of *Harmolita tritici*, the eggs often being placed outside the jointworm cell. Upon hatching, a larva that finds itself outside a cell of *H. tritici* does not seem to seek out a

larva of *H. tritici*, but begins at once to feed on the sap of the plant. This fact can easily be ascertained, as the larva is almost transparent and the chlorophyll appears very distinctly in the digestive tract. Numerous larvae apparently nearly ready to molt have been found that had not entered a cell of *H. tritici*, and had never fed on anything but plant sap. In such cases there was always a distinct little pocket in which the larva was resting and feeding. This observation certainly indicates that the larvae can, and in many instances possibly do, reach maturity, and during their existence feed on nothing but plant sap. These observations were made from a great number of dissections of both cage and field material.

Probably the majority of the eggs are placed in the jointworm cells, but rarely in direct contact with the larva of *Harmolita tritici*. Upon hatching, the larval parasites devour the host larva in a short time. They have often been found when they had just completed feeding on the Harmolita larva, and were still in the first instar and not ready to molt. In such cases they apparently proceed at once to feed on the plant sap. They seem to be able to migrate through the plant tissue for microscopical distances only.

Upon hatching, many larvae of *Eurytoma parva* find themselves in the cell with a larva of *Harmolita tritici* of the second instar, and proceed at once to devour it. The majority, perhaps, are placed on host larvae of the first instar. As previously stated, the adult female of *Eurytoma parva* seems to prefer the jointworm larvae in the first and second instars for oviposition, even though host larvae of the later instars are available.

The larvae of *Eurytoma parva* simply tear a hole in the body wall of the host larva with their mandibles, and suck out the contents of the body. After having apparently consumed the host larva they continue to occupy the jointworm cell and begin to feed on the plant sap. The evidence that the larvae of *E. parva* do feed upon the larvae of *Harmolita tritici*, and do not simply kill them in order to gain possession of the cell, is the fact that in all such cases no chlorophyll is seen in their bodies until after the host larva is consumed.

During this study approximately a thousand cells of *Harmolita tritici* have been opened which contained larvae of *Eurytoma parva* or had them in the immediate vicinity, and in only a few instances were the Eurytoma larvae found feeding on the larvae of *H. tritici* of the third or fourth instar. In the few exceptions two jointworm cells were very close together, with a very thin partition wall. The larva of *E. parva* apparently breaks accidentally through this wall in lacerating the plant tissue to secure more plant sap. In these instances the second jointworm larvae are killed and perhaps devoured, though this point has not been definitely established.

If several larvae of *Eurytoma parva* hatch in a single jointworm cell, a struggle ensues and the strongest individual apparently survives and takes possession of the cell. This is indicated by the occasional finding of several partially destroyed first-instar larvae of *E. parva* in a cell of *Harmolita tritici* with a strong, vigorous-looking individual of the same instar.

SUMMARY

At present *Eurytoma parva* is apparently one of the most important parasites of the wheat jointworm, *Harmolita tritici*.

It is both parasitic and phytophagous in habit. This is the first detailed observation made of this relationship for American Chalcidoidea.

Evidence has been produced which indicates that *Eurytoma parva* may reach maturity on plant sap alone.

In one instance a larva of the second instar of *Eurytoma parva* that had evidently consumed a larva of *Harmolita tritici* and had begun to feed on plant sap was reared to maturity upon a full-grown larva of *H. tritici*.

The observations detailed in this paper indicate that *Eurytoma parva* is gradually changing from a parasitic to a phytophagous habit.

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THE RATE OF PASSAGE OF FOOD THROUGH THE DIGESTIVE TRACT OF THE HEN[1]

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REVIEW OF LITERATURE

The earliest recorded attempt to determine the time required for food to pass through the gastrointestinal tract of hens or other birds is probably that of Weiske, reported in 1878 (*9*).[2] After Weiske had fed 2 geese on dandelion leaves for 8 days, he abruptly changed the ration to barley grains, and within 3 hours and 25 minutes he found the barley grains in the excrement of the birds, swollen but otherwise little altered in appearance. Within 3 hours more, that is, about 6½ hours after the feed was changed, he observed that the excrement consisted entirely of residues of the barley with no green feed. After the geese had been fed grain only for several days, this ration was discontinued and green feed only was given. The green feed first appeared in the feces of one bird after 1 hour and 40 minutes and in that of the other after 1 hour and 45 minutes. After 3 hours and 30 minutes the excrement of both birds consisted almost exclusively of residues of the green feed, with only scattered bits of the barley kernels.

Recently a few rather extended efforts have been made to gather digestion data on poultry by methods similar to those used with human beings. T. G. Browne (*2*) determined the time required for food to traverse the tract of hens by a study of the droppings. In his early experiments he used small colored seeds, such as those of red millet and clover, but these he found unsuitable because of the difficulty of identifying them in the feces. Bread dough colored with magenta, aluminum powder, gentian violet, or methylene blue was tried; but the fluids of the digestive tract seemed to take the stain and carry it forward faster than the solids passed, thus staining everything on the way and leaving the original material behind. It was found, however, that fluids pass more rapidly through the canal than solids and that the rate of the passage varies directly with the quantity of fluid consumed. White and black oats were finally used and were found to be easily distinguishable in the feces. According to Browne's report, oats fed when the crop is empty appear in the feces within 5 to 6 hours, the time varying usually not more than an hour. The crop filled with oats empties within 18 to 20 hours. An entire meal of oats is passed within 27 to 28 hours. In two experiments in which birds were killed 20 and 10 minutes after they had begun to eat oats, a few oat grains were found in the gizzard. Retention of feed in the crop varies with the amount consumed, the more fluid portions passing out first. Liquids do not pass entirely through at once, although some portion passes immediately into the intestine. Liquids are not retained in the gizzard.

[1] Received for publication Jan. 11, 1927; issued June, 1927.
[2] Reference is made by number (italic) to "Literature cited," p. 770.

Kaupp and Ivey (*4, p. 10; 5*) used practically the method discarded by Browne. A mash colored by lampblack or a dye was fed, and the time at which the stain was last seen, together with that at which it first appeared in the droppings, was noted. Neither methylene blue nor gentian violet was found to be satisfactory, because these dyes seemed to cause constipation, which was followed by diarrhea. Lampblack gave more satisfactory results. From the averages of their results on two to six birds of each kind, the writers summarize their observations as follows:

> Digestive processes of the fowl are rapid. The greatest rapidity is shown in the laying and in the growing fowl, food passing on an average of 3 hours and 52 minutes in the case of growing fowls and 3 hours and 46 minutes in the cases of the laying hens. Next in activity comes the adult hen not in laying condition, averaging 8 hours, and then the broody hen required an average of 11 hours and 44 minutes.

The valuable bulletin of E. W. Brown on the subject of digestion in poultry (*1*) gives some data on the retention of food in the crop, which may be summarized as follows: 30 gm. of corn fed in the morning into the empty crops of each of two fowls weighing 3 pounds, 5 ounces had entirely passed into the gizzard within 11 hours and 30 minutes and 12 hours and 15 minutes; the same quantity of corn fed the following morning had passed into the gizzard within 13 hours, and 13 hours and 30 minutes; 35 gm. fed at night had entirely disappeared within 15 hours, and 15 hours and 30 minutes; 50 gm. fed at 2 p. m. disappeared in 24 hours, 15 minutes and 24 hours, 25 minutes. These observations are said to be typical of a large number made with several fowls. The period of retention of oats in the crop was essentially similar. The figures indicate that larger amounts of feed require a longer time to leave the crop and individual birds of equal weight show slight variations.

Schwarz and Teller (*7*) tested the retention of feed in the crop, using eight hens and feeding to them four different grains, namely, wheat, barley, maize, and oats. Before the experiments began the hens were allowed to fast for 24 hours. Thirty grams of the grain was then fed to them and no other feed was given during the experiment, though water was always at hand. The presence of grain in the crop was determined by palpation. At first the palpation test was tried once an hour, but after the fifth time once a half-hour. The time that the feed remained in the crop varied greatly between different individuals and even between different tests on the same bird; but in general when 30 gm. of grain was fed the crop emptied itself in about 11 to 12 hours. The average length of time for the four grains was: Wheat, 11 hours; barley, 10 hours, 50 minutes; maize, 11 hours, 50 minutes; and oats, 12 hours, 20 minutes. The actual variation observed was from 5 to 15 hours. So wide a variation in the individual figures detracts from the significance of the averages. While grain is in the crop it absorbs moisture and swells. The effect of this absorption was studied by removing wheat grains from the crop at the end of 4, 6, and 8 hours and comparing their weight with that of similar grains which had not been ingested. The gain in weight of wheat staying in the crop for 4 hours was 11.43 per cent; of that staying 6 hours, 32.69 per cent; and of that staying 8 hours, 36.71 per cent.

Quite recently Steinmetzer (*8*) has reported observations with Röntgen-ray apparatus. Uniform pellets were prepared from a

definite weight of the feed (groats) mixed with an equal weight of barium sulphate and a little water, the barium sulphate allowing the position of the feed at any time to be seen and to be photographed. The hens fasted for 24 hours before the experiments. At the time of the introduction of the pellets and immediately following, observations were made under the rays at first once in 5 minutes, then once in a quarter of an hour, and finally once an hour. Both the crop and the gizzard were visible when empty and their movements in response to the feeds could be watched. As the pills were dropped in, some of them slipped directly past the crop into the gizzard, though the majority remained in the crop for a time. All stopped for about 0.5 to 1 minute at about 1 to 2 finger-widths from the shadow of the gizzard and then slipped into the gizzard. This pause evidently indicated a short stop in the glandular stomach. In general, the passage of the feed from part to part was, in this case, quite rapid, as is shown by the following average figures of the intervals after feeding at which the food appeared in and disappeared from the segments.

Segment	Interval after feeding	
	First appearance of the food	Final disappearance of the food
Crop	0	2 hours.
Gizzard	0.5–1.0 minute	2 hours 15 minutes.
Small intestine	15 minutes	3 hours.
Large intestine	2 hours	4 hours.

The author thus summarizes his conclusions (*8, p. 505*):

> The passage of the food through the gastrointestinal canal of the cock is normally exceedingly rapid; in the case of the food used in our experiments in pill form, in 4 hours. The feed remains in the crop, the gizzard, and the large intestine for equal intervals of time (in our experiments an average of 2 hours), in the small intestine about half as much longer (in our experiments an average of 3 hours). The gizzard is without movement when it is in an empty condition, but begins rhythmic movements as soon as food passes to it. The glandular stomach was always found to be empty except for the short moments when from time to time portions of the food passed through it (0.5 to 1 minute). The behavior of the crop was peculiar. Although the attempt was made in all the experiments, no rule could be discovered as to when the pill would fall into the crop and when not.

Data obtained with these small amounts of treated feed in pill form are instructive, though they can not be applied directly to the conditions of practical feeding. One experimental series with twice the amount of feed gave about twice as long intervals.

In connection with a careful study of the movement and digestion of starch broth in the different parts of the digestive tract of pigeons, Mangold (*6*) noted that in the cloaca and throughout the length of the intestine, indigestible hulls of grain were always to be found, together with small stones from the gizzard, even after the pigeons had been fed only starch broth for 3 to 6 days. This fact indicates that such indigestible pieces may remain for a long time in the gizzard even in large amounts, to be passed later into the intestine.

In the experiments to be reported here, a study was made of the dry weight and the moisture content of the material found in the different segments of the digestive tract of hens at various intervals

after the ingestion of three types of feed in common use, namely, whole corn, ground corn, and ground corn combined with a protein concentrate.

PLAN OF THE EXPERIMENTS

After a fast of 60 hours, during which time water was accessible but not food or grit, hens were individually fed 50 gm. of the feed to be observed and were then kept in separate cages, without access to food, for different intervals of time. Any feed remaining uneaten after 30 minutes was force fed. In the first experiment water was kept before the birds after the feeding. In the second experiment 75 c. c. of water was given to each bird in connection with the feeding, 50 c. c. being mixed with the ground feeds and the rest being introduced into the crop by means of a pipette. For the whole-corn group, 75 c. c. of water was introduced by pipette after the feeding of the corn. At the end of the desired time the birds were killed, the digestive tracts immediately removed—care being taken not to disturb the contents—and the contents of the several segments placed in weighed evaporating dishes and weighed as promptly as possible. The sections selected were: (1) Crop and tubes down to the proventriculus, (2) gizzard and proventriculus, (3) small intestine from gizzard to caeca, (4) caeca, and (5) large intestine including cloaca. In the first experiment (4) and (5) were combined. The excreta were also collected, dried, and weighed.

The contents of each segment were dried at 105° C. and the moisture determined. To insure greater accuracy in finding the weight of the feed in the gizzard, the whole material from the gizzard was incinerated in the muffle and the stones were separated as completely as possible from the ash and their weight was taken as the weight of the grit. The dry weight of the contents minus the weight of the grit is given as the dry weight of feed in the gizzard.

RATE OF PASSAGE OF FOOD AS INDICATED BY THE WEIGHT OF DRY MATTER IN SEGMENTS OF THE DIGESTIVE TRACT AT VARIOUS INTERVALS AFTER FEEDING

EXPERIMENT 1: WHOLE CORN

In the first experiment 50 gm. of whole corn was fed to each hen and the birds were killed at intervals of 1, 2, 4, 7, 12, 15, 24, and 48 hours afterward and examined. For comparison, another hen was killed and examined in the same way after a fast of 60 hours following the usual green feeds. The weights of dry matter found are given in Table 1.

The data indicate that the corn left the crop rather rapidly at first and then more slowly while gizzard digestion was going on, more than 12 hours being required for it to pass entirely from the crop. In the hen which was killed at the end of one hour three corn kernels were found which had not yet reached the crop. From the data in Table 1 it seemed that by the end of 15 hours the amount of dry material in the crop, gizzard, and large intestine had returned approximately to a fasting level. The amount in the small intestine showed no regular change. The average amount in the small intestine was 3.87 gm. and in the large intestine, 1.36 gm.

TABLE 1.—*Weight of dry matter in sections of the digestive tract and in the excreta of hens fed whole corn; experiment 1*

Hours after feeding	Weight in grams of contents of—					Weight of feces (grams)	Remarks
	Crop	Gizzard		Small intestine	Caeca and large intestine		
		Dry matter	Dry matter not grit				
1	37.66	16.84	8.54	3.50	1.26	0	Much whole corn in tubes, as well as crop; 6 whole kernels in gizzard.
2	34.88	22.31	9.18	4.84	1.49	0	1 whole kernel in gizzard.
4	29.21	16.41	6.14	4.14	2.13	2.81	Much water in crop; 1 whole kernel in gizzard.
7	19.25	24.21	12.81	4.49	2.50	4.07	4 whole kernels in gizzard.
12	22.21	31.77	10.38	2.92	1.26	4.54	25 whole kernels in gizzard.
15	1.87	16.60	6.72	4.97	1.36	6.88	Crop showed only secretion; no whole kernels in gizzard.
24	.04	15.93	5.14	2.88	.84	11.99	No appearance of corn anywhere.
48	.03	12.79	5.84	2.52	1.15	18.24	No appearance of corn.
60 (control)	.45	18.25	9.94	4.56	.29		Straw in crop; grass in gizzard.
Average				3.87	1.36		

EXPERIMENT 2: WHOLE CORN, GROUND CORN, AND GROUND CORN PLUS TANKAGE

In Experiment 2 the hens were fed whole corn, ground corn, and a mixture of ground corn and tankage in the proportion of 4 to 1. The experiment was carried out as before with the exception of the intervals allowed after feeding and before killing the birds. In order to obtain more complete information on the later periods of digestion without multiplication of birds, it was decided to obtain data for the time at which the last of the feed left the crop and at intervals of 1, 2, 3, and 4 hours thereafter. The time at which the crop emptied was judged by external manipulation, and the hens were killed accordingly. This method of estimating the condition of the crop did not prove satisfactory for the kind of study desired. Hence the data will be considered entirely with regard to the interval after feeding and without regard to the time after the crop appeared to be empty. The weights of dry matter obtained in experiment 2, arranged on that basis, are given in Table 2.

In a consideration of this larger collection of data, the great irregularity in the results is at first most striking. Wide variations among the individual birds occur which can not be attributed to the difference in the intervals which elapsed after feeding. For instance, one bird of the whole-corn group had 34 kernels (small ones) in the crop after 23 hours, and one of the ground-corn group had 5 gm. of dry matter in the crop at the end of 35 hours. This last amount is nearly the same as was found in a hen of the same group after 12 hours. One factor which partly explains the irregularities and at the same time complicates the interpretation of the data is the uncertainty as to the amounts of the secretions present. When a crop contains nothing but its own secretions it may show nearly 2 gm. of dry matter. We have no means of judging the quantity of secretion present in the other sections of the digestive tract. After the longer periods of digestion the secretions probably make up larger proportions of both the dry matter and the moisture present.

TABLE 2.—*Weight of dry matter in sections of the digestive tract and in the excreta of hens fed whole corn, ground corn, and ground corn and tankage; experiment 2*

WHOLE CORN

Hours after feeding	Weight in grams of contents of—						Weight of feces (grams)	Remarks
	Crop	Gizzard		Small intestine	Caeca	Large intestine		
		Dry matter	Dry matter not grit					
11.3	0.56	8.42	7.85	4.04	.....	[a] [b] 0.65	3.05	Crop 2 whole kernels. Gizzard 15 whole kernels.
12.8	.45	11.41	6.10	3.91	1.21	.43	6.00	Crop 1 whole kernel. Gizzard 3 whole kernels.
15.4	.....	7.02	[b] .24	1.64	.63	.39	5.27	Crop 1 whole kernel. Gizzard no whole kernels.
19.7	.91	9.74	6.84	2.57	.63	.89	4.22	Crop no whole kernels. Gizzard no whole kernels.
20.2	.76	19.02	8.19	2.09	.18	.26	6.01	Crop 1 whole kernel. Gizzard 5 whole kernels.
21.1	.....	10.03	4.94	2.05	[c]	.23	4.94	Crop no whole kernels. Gizzard 2 whole kernels.
21.1	.....	13.22	3.39	1.37	.78	.20	4.28	Crop no whole kernels. Gizzard no whole kernels.
23.3	7.08	16.54	4.87	2.40	.73	.19	3.77	Crop 34 whole kernels. Gizzard 2 whole kernels.
Average	.....	.....	6.03	2.51	.69	.37	.....	

GROUND CORN

11.8	4.33	8.98	6.23	5.08	0.59	0.57	3.06
13.5	3.73	20.60	5.46	3.53	1.66	1.03	7.87
15.0	2.72	10.28	4.84	2.17	.66	.25	4.05
16.1	14.14	12.64	4.78	3.34	.78	.89	1.54
18.5	.63	11.88	5.33	2.52	.38	.14	5.38
34.8	4.97	16.82	7.91	3.35	.71	.90	7.04
Average	.....	.....	5.76	3.33	.80	.63	.....

GROUND CORN AND TANKAGE

11.6	2.71	13.72	6.64	5.53	0.34	0.38	5.87
13.2	2.58	17.72	5.53	4.23	.33	[c]	5.47
19.4	.39	15.48	7.36	3.86	.77	.74	.99
20.5	1.25	10.12	4.86	2.26	.68	.48	7.12
23.2	.23	20.20	5.36	1.97	.51	[c]	4.90
24.2	.86	9.82	6.17	2.24	.34	.67	6.24
25.1	.37	15.40	5.90	4.45	1.00	1.22	3.90
26.1	.90	10.66	3.58	3.84	.39	[c]	4.71
Average	.....	.....	5.68	3.55	.55	.70	.....

[a] Large intestine + caeca. [b] Not included in the average. [c] Lost.

Some interesting and significant facts are brought out if we consider not individual birds but the conditions found during periods of several hours each. In Tables 3 and 4 the data of experiments 1 and 2, respectively, have been arranged to permit of such a study. The periods selected are those covered by the hours 1 to 4 (experiment 1), 5 to 10 (experiment 1), 11 to 14 (experiments 1 and 2), 15 to 21 (experiments 1 and 2), 22 to 28 (experiments 1 and 2), and more than 30 (experiments 1 and 2).

TABLE 3.—*Average weight of dry matter in sections of the digestive tract of the hens during successive periods after feeding; feed, whole corn; experiment 1*

Hours after feeding	Weight in grams of contents of—			
	Crop	Gizzard	Small intestine	Caeca and large intestine
1–4 [a]	33.9	8.0	4.2	1.6
7	19.3	12.8	4.5	2.5
12	22.2	10.4	2.9	1.3
15	1.9	6.7	5.0	1.4
24	.04	5.1	2.9	.8
48	.03	5.8	2.5	1.1
60 (control)	.5	9.9	4.6	.3

[a] 3 birds; in each of the other cases, 1 bird.

TABLE 4.—*Average weight of dry matter in section of the digestive tract of hens during successive periods after feeding; feed, whole corn, ground corn, and ground corn and tankage; experiment 2*

Hours after feeding	Weight in grams of contents of—														
	Crop			Gizzard			Small intestine			Caeca			Large intestine		
	Whole corn	Ground corn	Corn and tankage	Whole corn	Ground corn	Corn and tankage	Whole corn	Ground corn	Corn and tankage	Whole corn	Ground corn	Corn and tankage	Whole corn	Ground corn	Corn and tankage
11–14	[a] 0.5	[a] 4.0	[a] 2.6	[a] 7.0	[a] 5.8	[a] 6.1	[a] 4.0	[a] 4.3	[a] 4.9	[b] 1.2	[a] 1.1	[a] 0.3	[b] 0.4	[a] 0.8	[b] 0.4
15–21	[a] .8	[c] 5.8	[a] .8	[d] 4.7	[c] 5.0	[a] 6.1	[d] 1.9	[c] 2.7	[a] 3.0	[c] .6	[c] .6	[a] .7	[d] .4	[c] .6	[a] .4
22–28	[b] 7.1		[c] .6	[b] 4.9		[c] 5.3	[b] 2.4		[a] 3.1	[b] .7		[c] .6	[b] .2		[a] .9
35		[b] 5.0			[b] 7.9			[b] 3.4			[b] .7			[b] .9	

[a] 2 birds. [b] 1 bird. [c] 3 birds. [d] 5 birds. [e] 4 birds.

From a consideration of these tables, representing the two experiments, the following conclusions seem to be justified:

Crop. All whole corn leaves the crop by the end of 12 to 15 hours. Ground corn leaves the crop more gradually, with the result that when ground corn is fed a much longer time is required for the crop to become empty. Ground corn and tankage mixed in the ratio of 4 to 1 leave the crop more slowly than whole corn but more rapidly than ground corn alone.

Gizzard. The amount of dry matter in the gizzard in addition to the grit is larger when there is feed in the crop than when there is none, but otherwise it does not vary greatly. Nor does it vary with the three types of feed used.

Small intestine. The amount of dry matter in the small intestine is the same for the three feeds, and is somewhat higher during the first 15 hours after feeding than it is later.

Large intestine and caeca. The amount of dry matter in the large intestine and the caeca probably does not change very much during digestion, though it may rise somewhat 5 to 10 hours after feeding. (See Table 1.) The figure for the control hen of experiment 1 indicates that it may fall markedly during a prolonged fast.

MOISTURE IN THE DIGESTIVE TRACT OF HENS

The data collected reveal some interesting facts with regard to the degree of moisture present in the different sections of the digestive tract of the hen. Table 5 shows the percentages of moisture found in experiment 1 and Table 6 those found in experiment 2.

TABLE 5.—*Percentage of moisture in the contents of sections of the digestive tract of hens fed whole corn; experiment 1*

Hours after feeding	Percentage of moisture in the contents of—			
	Crop	Gizzard	Small intestine	Caeca and large intestine
1	23.2	43.9	84.7	77.7
2	17.9	35.9	86.4	83.1
4	49.9	36.8	85.0	82.4
7	38.7	47.8	83.2	79.6
12	20.6	32.4	85.1	80.9
15	52.8	46.1	82.0	81.9
24	95.1	34.5	82.5	92.9
48	95.0	44.7	83.9	86.7
60 (control)	74.5	47.7	76.8	76.1
Average	----------	41.1	83.3	82.4

TABLE 6.—*Percentage of moisture in the contents of sections of the digestive tract of hens fed whole corn, ground corn, and ground corn and tankage; experiment 2*

WHOLE CORN

Hours after feeding	Percentage of moisture in the contents of—				
	Crop	Gizzard	Small intestine	Caeca	Large intestine
11.3	69.7	57.3	82.6	[a] 80.2	----------
12.8	67.6	47.6	82.2	69.0	79.2
15.4	----------	54.6	75.0	80.3	86.2
19.7	80.1	58.2	84.4	79.5	77.7
20.2	88.6	45.4	85.1	77.2	82.6
21.1	----------	43.4	86.8	[b]	80.1
21.1	----------	34.9	82.6	71.1	81.5
23.3	54.4	40.8	84.0	70.9	72.0
Average	----------	47.8	82.8	74.7	79.9

GROUND CORN

11.8	61.4	56.6	82.2	69.7	77.0
13.5	60.2	31.1	83.9	73.2	67.6
15.0	62.3	50.4	82.4	78.5	78.6
16.1	53.5	48.4	80.5	69.5	74.6
18.5	78.5	39.5	83.3	79.9	75.9
34.8	61.5	46.5	85.8	80.5	77.4
Average	----------	45.4	83.0	75.2	75.2

GROUND CORN AND TANKAGE

11.6	67.2	47.0	80.9	78.1	78.7
13.2	74.2	34.4	83.2	76.8	[b]
19.4	68.9	43.4	86.8	80.8	85.0
20.5	65.6	46.4	84.5	71.7	81.2
23.2	91.3	30.9	80.1	80.9	[b]
24.2	74.6	54.9	82.6	79.8	82.1
25.1	77.7	45.3	81.6	77.5	82.6
26.1	74.7	41.7	81.8	82.4	[b]
Average	----------	43.0	82.7	78.5	81.9

[a] Caeca+large intestine; not included in the average. [b] Lost.

TABLE 7.—*Amount of water in the crop and gizzard, and ratio of moisture to dry matter not grit in gizzard*

EXPERIMENT 1: WHOLE CORN

Hours after feeding	Water in—		Ratio of moisture to dry matter not grit in gizzard
	Crop (grams)	Gizzard (grams)	
60 (fast)	1.3	16.7	1.7 : 1
1	11.3	13.2	1.6 : 1
2	7.6	12.5	1.4 : 1
4	[a]29.1	9.5	1.6 : 1
7	12.1	22.2	1.7 : 1
12	5.8	15.2	1.5 : 1
15	2.1	14.2	2.1 : 1
24	.8	8.4	1.6 : 1
48	.6	10.3	1.8 : 1

EXPERIMENT 2: WHOLE CORN

11	1.3	11.3	1.4 : 1
13	.9	10.4	1.7 : 1
15	[a]2.2	8.4	[b]35.1 : 1
20	3.7	13.6	2.0 : 1
20	5.9	15.8	1.9 : 1
21		7.7	1.6 : 1
21		7.1	2.1 : 1
23	8.5	11.4	2.3 : 1

EXPERIMENT 2: GROUND CORN

12	6.9	11.7	1.9 : 1
14	5.7	9.3	1.7 : 1
15	4.5	10.5	2.2 : 1
16	16.3	11.9	2.5 : 1
19	2.3	7.8	1.5 : 1
35	8.0	14.6	1.8 : 1

EXPERIMENT 2: GROUND CORN AND TANKAGE

12	5.6	12.2	1.8 : 1
13	7.4	9.3	1.7 : 1
19	.9	11.9	1.6 : 1
21	2.4	8.8	1.8 : 1
23	2.4	9.0	1.7 : 1
24	2.5	12.0	1.9 : 1
25	1.3	12.7	2.2 : 1
26	2.7	7.6	2.1 : 1

[a] Much visible water. [b] Very little food residue.

The quantity of moisture in the crop is very variable, and in these experiments shows no relation to the type of feed eaten or to the interval after feeding. In experiment 1, where water was kept before the birds all of the time, the amount in the crop may have been affected by the time after drinking. In some cases water was distinctly evident in the crop, either as water drunk or as secretion. In the other sections, aside from the gizzard, the percentage of moisture remained nearly constant. In the gizzard the moisture content was rather variable, though less so than in the crop. An inspection of Tables 5 and 6 shows strikingly that the percentage of moisture in the gizzard is not a reflection of that in the crop. For example, two birds in experiment 1 each showed 48 per cent moisture in the gizzard, but the amount in the crops of the same birds varied as widely as from 75 to 39 per cent; one bird of the corresponding group in

experiment 2 which had 45 per cent moisture in the gizzard, showed 89 per cent in the crop. The same lack of uniformity in moisture content of crop and gizzard is apparent if one considers the actual amount of moisture in these organs instead of the percentages. The amount of moisture in the crop seems to be absolutely independent of its other contents and that in the gizzard is usually about 1.5 to 2 times the amount of dry matter not grit present (Table 7).

The percentages of moisture present in the several sections of the digestive tract may be summarized thus: (1) In the crop an indefinitely variable amount; (2) in the gizzard about 30 to 60 per cent (the general average for the four groups was 44.2 per cent); (3) in the small intestine about 82 to 86 per cent (the average in each group was 83 per cent); (4) in the caeca 70 to 80 per cent (the general average for three groups was 76.4 per cent); and (5) in the large intestine 75 to 85 per cent (the general average for three groups was 78.9 per cent). The average percentage in the caeca and large intestine of the hens in experiment 1 was 82.4 per cent. The percentage was remarkably constant in the small intestine. As was to to be expected, the small intestine showed the highest percentage generally and the large intestine next. The moisture content of the caeca was slightly lower than that of the large intestine. The moisture content of the gizzard was distinctly low.

GRIT IN THE GIZZARD OF HENS

E. W. Brown (*1*) undertook a study of the utilization of sand by poultry. Comparisons were made of the SiO_2 consumption and excretion, and in some cases the gravel found in the crop and gizzard was weighed and examined. With free access to gravel the amount of both the ingestion and excretion varied widely with the individual birds and with the type of feed. The values were considerably higher on an oat diet than on a corn diet. Under such conditions the excretion sometimes reached 6 gm. of sand per day; but when no grit was available, the excretion fell to a low figure.

T. G. Browne (*2*), performing experiments in which sand and shot were mixed with the food when the normal amount of grit was otherwise provided, found that this excess sand and shot appeared in the feces in the 5-hour interval usual for food. On the other hand, he found the gizzard rarely if ever free from sand and pebbles even after the birds had been without access to grit for six weeks.

A few observations on the amount of grit in the gizzard were made by Buckner and Martin (*3*) in connection with a study of the formation of bone and egg shell from different mineral sources. After eight months the lot receiving nothing but grain and tankage showed 12.6 gm. of grit in the gizzard.

Kaupp and Ivey (*4*, *5*) made similar observations, reporting grit present in the gizzard even after a year without opportunity for renewal, one hen showing 5.89 gm. of grit after 365 days. The birds still appeared healthy, indicating that the retained grit was sufficient to grind the feed.

All of these observers have found grit constantly present in the gizzard, but in very different quantities. Their work also gives evidence that grit may normally be found in all sections of the digestive tract and in the excreta. It appears, however, that when there is no opportunity for the chicken to pick up grit, whatever is in the gizzard is retained there for a long time.

In the experiments reported here no attempt was made to estimate the amount of grit or sand in any portion of the tract except the gizzard. The probable presence of sand is, however, one of the factors contributing to the complexity of the problem and may explain in part the irregularities of the results obtained. In the gizzard the grit makes up so large and so variable a part of the total dry matter that a rough determination of the amount was made by ashing at low red heat the total contents of the gizzard, sifting out with a 40-mesh sieve the fine powdery ash, and weighing the residue as grit. The weights so obtained are given in Table 8. The birds had all been without access to grit for 60 hours previous to the beginning of the experiment.

TABLE 8.—*Grit content of the gizzard of hens used in experiment 1 and experiment 2*

Experiment 1		Experiment 2					
Whole corn		Whole corn		Ground corn		Corn and tankage	
Hours after feeding	Grit (grams)	Hours after feeding	Grit (grams)	Hours after feeding	Grit (grams)	Hours after feeding	Grit (grams)
60 (fast)	8.31	11	0.57	12	2.75	12	7.08
1	8.30	13	5.31	14	15.14	13	12.19
2	13.13	15	6.78	15	5.44	19	8.12
4	10.27	20	2.90	16	7.86	21	5.26
7	11.40	20	10.83	19	6.55	23	14.84
12	21.38	21	5.09	35	8.91	24	3.65
15	9.88	21	9.83			25	9.50
24	10.80	23	11.67			26	7.08
48	6.95						
Average	11.16		6.62		7.77		8.47

Table 8 shows a rather wide variation in the amount of grit in the gizzards of the hens. In one case there was as little as 0.57 gm. and in another case as much as 21 gm.; but in general the amount ranged from 3 to 15 gm. No connection was observed between the amount of grit present and any other factor or condition.

SUMMARY

Determinations have been made of the amount of dry matter contained in the various segments of the digestive tract of hens at different intervals after the ingestion of 50 gm. of whole corn, ground corn, or a mixture of ground corn and tankage in the ratio of 4 to 1.

The results indicate a wide variability in the rate of passage of the food through the hen. In most cases, however, the whole corn had left the crop by the end of 12 to 15 hours. The ground corn remained in the crop distinctly longer than the whole corn and somewhat longer than the mixture of corn and tankage. The amount of dry matter in the other sections of the digestive tract did not differ notably for the three kinds of feed used. The amount of dry matter in the gizzard in addition to the grit was larger while food remained in the crop than later, and to a less extent the same was true of the small intestine. Otherwise the amount of dry matter in the intestines was fairly constant.

The percentage of moisture in the contents of the crop showed a wide variation, apparently independent of the kind of feed eaten and of the time after feeding. The percentage of moisture within the gizzard was low and rather variable, being about 30 to 60 per cent, with an average of 44.2 per cent. The amount was not a reflection of the amount in the crop, but was usually about one and one-half to two times that of the dry matter not grit present. The percentage of moisture within the small intestine was high and remarkably constant at about 82 to 86 per cent, with an average of 83 per cent. That in the caeca was about 70 to 80 per cent of the total contents, with an average of 76.4 per cent; and that in the large intestine was about 75 to 85 per cent, with an average of 78.9 per cent.

The amount of grit found in the gizzard varied widely, the usual range being from 3 to 15 gm. No connection was observed between the quantity of grit present and any other factor or condition.

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SOME FACTORS OTHER THAN BACTERIA THAT INFLUENCE THE BODY OF ARTIFICIAL BUTTERMILK [1]

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INTRODUCTION

"The practice of adding sodium citrate to milk used as infant food," according to Van Slyke and Bosworth (*3*)[3] "has been common for many years. It has found application especially in the treatment of certain types of 'feeding-cases' in which untreated milk, after entering the stomach, forms abnormally large chunks of tough curd, shown by Talbot to consist of casein * * *. Empirical practice has shown that this abnormal curdling of milk may, to some extent, be modified or controlled by the addition of sodium citrate at the rate of 1 to 2 grains per ounce of milk."

To explain the mechanism of this practice on a chemical basis, those investigators conducted some experiments in which they showed that when sodium citrate is added to normal milk the time of coagulation is increased. The curd increases in softness with an increase in the amount of added citrate, and when as much as 0.4 gm. per 100 c. c. of milk is added, coagulation does not take place. They have also shown that there is an increase in the quantities of soluble calcium, magnesium, and phosphorus, and that the increase in soluble calcium is mainly at the expense of the casein molecule.

Later, Bosworth (*1*, *2*) published several papers dealing with the question of milk for infant feeding. He studied human milk and determined its probable chemical composition in the same manner that cow's milk was formerly studied by Van Slyke and himself. He reports that when rennet is added to it human milk will give a small amount of finely divided precipitate. The difference in the behavior of human milk from that of cow's milk is explainable, according to Bosworth, by the difference in the base content of both milks. He found a 7.2 gm. equivalent of calcium for each 10 gm. equivalent of sod iumand potassium in human milk, which is in contrast to a 13.2 gm. equivalent of calcium for each 10 gm. equivalent of sodium and potassium, found in cow's milk. Bosworth is inclined to believe that the casein of human milk is in the form of calcium potassium caseinate. In other words, the addition of sodium salts to cow's milk results in the prevalence of sodium and potassium over calcium and magnesium, thus changing the calcium caseinate of cow's milk to calcium sodium caseinate or simply to sodium caseinate, which resembles the casein of human milk in its characters.

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[2] Grateful acknowledgement is made to the International Association of Milk Dealers for a grant in aid of this investigation; and to Prof. J. M. Sherman for helpful advice and encouragement throughout the work.

[3] Reference is made by number (italic) to "Literature Cited," p. 784.

Bosworth's investigations and conclusions suggested to the writer the possibility of improving the body of commercial buttermilk made in this country from cow's skimmed milk by the addition to it of a certain quantity of a sodium salt. The manufacturers of this product have always experienced difficulty in preventing wheying off, and attempts to obtain a sure and practical method of manufacturing which would eliminate daily variations in the quality of the product have not been successful. Present methods of manufacturing vary with the plants and are largely empirical. The purpose of the investigation herein discussed was to study the possibility of developing, on a scientific basis, a method of manufacturing buttermilk which would eliminate the daily variations in the quality of that product. Such a venture is important from an economic as well as from a scientific point of view.

PROCEDURE AND TECHNIC

Given two different samples of buttermilk, how can one determine the quality of each more reliably and accurately than by the ordinary judging method? How can the inherent qualities of each sample be transformed into tangible elements, comparable even within narrow limits? That was the primary problem to be solved in this study. The value of the conclusions depends to a large extent on the accuracy with which the quality of each sample has been determined.

It was recognized in the beginning that there are in general three main characters which largely determine the quality of buttermilk. These are: (1) The texture of the product, i. e. the size and arrangement of the particles which make up its body; (2) the consistency of the product, i. e. the manner in which its particles adhere or are cemented together; and (3) the flavor of the product.

In this study the amount of the whey liberated was estimated in all instances and was taken into consideration before any conclusion was drawn.

The texture of the product was determined by the weight of particles above a certain arbitrary size. The separation of the particles into two classes was done by sedimentation. Three hundred cubic centimeters of water were measured into a 500 c. c. Erlenmeyer flask, into which 100 c. c. of the broken coagulum was poured slowly and carefully, as well as 50 c. c. of water, used to rinse the measuring cylinder. The object of adding water was to destroy the viscosity of the buttermilk as far as possible so that the lumps might settle freely. The flask was then rotated very carefully 10 times in order to mix its contents, and allowed to stand for one minute, so that the lumps could settle in the order of their coarseness. At the end of one minute the supernatant, and the the settling lumps, were poured over a Buchner funnel. In addition the flask was rinsed with 50 c. c. of water and the resulting contents poured into the funnel. This process separated the particles into two groups, the fine particles which passed through the openings, and the lumps which were retained on the funnel, from which they were washed with a gentle stream of water into a previously weighed, dry square of cheesecloth. The cheesecloth and its contents were allowed to dry for 24 hours in the air, when the weight of the dry lumps was determined.

The procedure is simple. It is difficult, however, to obtain accurate results with it, owing to the perishability of the lumps. The

strictest uniformity is necessary, therefore, in breaking up the coagulum and in the subsequent handling such as pouring and rotating. When carefully performed, the procedure may be said to be accurate to within 10 per cent.

The consistency of the product obtained from this process was measured by recording the time it took a plunger to travel a prescribed distance downward through a column of the product, on the theory that the consistency of a body is proportional to the resistance that body offers to a shearing force applied to it. A glass cylinder (C) was filled with the sample to a certain mark, and the plunger was placed so that its upper side was just below the surface, and allowed to sink. The more viscous the product, the longer it took the plunger to travel the same distance. The best disposition was found to be the one represented in Figure 1. The shearing force π is equal to the weight p of the plunger P, minus the sum $p'+p''$ where p' is the weight of the counterweight P', and p'' the weight of a volume of the product equal to the volume of the plunger.

$$\pi = p - (p' + p'')$$

The difference $p-(p'+p'')$ may be varied at will so that the sinking velocity of P may be reduced to the best advantage. In the apparatus used π was such that it took P 10 seconds to travel the same distance (l) in an 18 per cent gelatin solution of a specific gravity equal to 1.038 at 30° C.

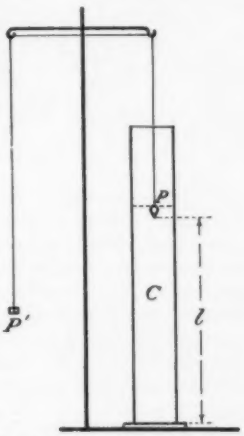

FIG. 1.—Outline of apparatus used to determine the consistency of buttermilk: C, glass cylinder; P, plunger; P', counterweight; l, distance traveled by the plunger

While this method of determining viscosity may give very accurate results with liquids of homogeneous character, its accuracy in testing the viscosity of buttermilk has been found to depend to a great extent on the texture of the sample of buttermilk. The larger the lumps, the greater, usually, was the viscosity recorded. This is to be expected, as those lumps include a large amount of water, so that the volume V of particles per cubic centimeter in Einstein's formula ($n_s = n_m\ (1+2.5\ V)$) will be increased. As a consequence therefore, the figures representing viscosity may not add to the information obtained from the weight of the lumps. Nevertheless, viscosity determination has been found of great value in instances where a relatively coarse body has a very poor consistency, as in the case of buttermilk made from milk heated to 145° F. for 30 minutes, at which temperature the body gets too weak to measure viscosity. A viscosity of 20 or slightly above was found to correspond to the optimum results.

The method usually followed in this study to test the effect of a chemical in influencing changes in the body of artificial buttermilk was to weight 0.1, 0.2, 0.3, 0.4, and 0.5 per cent portions of the chemical into 1-liter Erlenmeyer flasks to each of which 750 c. c. of raw skimmed milk was also added. The flasks were plugged with cotton,

heated in an Arnold steam sterilizer to 100° C., and held at that temperature for 15 minutes. They were then cooled to 25° C. or below and inoculated with a pure culture of *Streptococcus lactis*, after which they were usually incubated at room temperature which varied occasionally from 21° to 28°, but was most of the time in the neighborhood of 25°. At the end of 24 hours, during which the flasks were inspected occasionally, the coagula were broken by rotating 30 times, 15 clockwise and 15 anticlockwise, care being taken to observe as much uniformity as possible. One hundred cubic centimeters of the contents was then removed from each flask and the texture determined. The remaining portion served to determine the viscosity. A sixth flask to which no chemicals were added served as a control.

This general procedure was modified according to the object of the experiment.

In all instances the flasks were judged before and after the coagulum was broken and a record made of the quantity of free whey, and the character and flavor of the coagulum. The figures obtained by the methods above described were then compared. A stop watch was used whenever it was necessary to make time records.

PRELIMINARY EXPERIMENTS

Before the effects of the various salts were studied it was found necessary to determine how certain factors like the quantity of inoculum, the temperature to which the milk is heated, and the temperature at which it is incubated, would affect the quality of the product. If the effect due to a chemical is to be studied, those factors should be controlled, and the variations which they produce, once determined in degree and direction, should be eliminated. It was believed also that such variations might be utilized for the improvement of the product.

EFFECT OF QUANTITY OF INOCULUM ON QUALITY OF BUTTERMILK

It is not possible to state definitely what quantity of inoculum should be added to a given quantity of milk to get the best results. Theoretically, the quantity of inoculum should depend largely on the condition of the culture used for inoculation; the more virulent the culture, the smaller should be the quantity of inoculum added. In this work a highly virulent 24-hour-old culture was used most of the time, so that 0.05 c. c. of culture added to a liter of milk usually produced coagulation in from 12 to 15 hours. The results obtained are summarized in Table 1.

TABLE 1.—*Effect of quantity of inoculum on quality of buttermilk*

Flask No.	Inoculum per 750 c. c. of milk (c. c.)	Quantity of undesirable lumps (mgm. per 1,000 c. c.)		Viscosity in seconds		Percentage acidity as lactic acid	
		At 24° C.	At 28° C.	At 24° C.	At 28° C.	At 24° C.	At 28° C.
1	0.03	7	493	20.0	23.8	0.685	0.685
2	.1	8	694	24.1	28.6	.710	.690
3	.2	17	787	24.4			
4	.3		840	24.8		.720	.710
5	.4	21	1,302	25.2	28.1		
6	.5	29	1,485		29.4	.73	.720

Table 1 shows that, other things being equal, an increase in the quantity of inoculum causes an increase in the lump content and the titratable acidity of the product. Similarly an increase in the quantity of free whey and the firmness of the coagulum was observed to accompany an increase in the quantity of inoculum. The relative increase in these various factors at both temperatures used is in close agreement, but the magnitude of that increase becomes more and more important as the temperature of incubation is increased, so that for practical purposes the following conclusion may be drawn: At a low incubation temperature, the quantity of inoculum is a minor factor, but it increases in importance as the incubation temperature increases. A large dose of inoculum tends to render the product lumpy and more acid.

EFFECT OF THE DEGREE TO WHICH THE MILK IS HEATED, AND OF INCUBATION TEMPERATURE ON BUTTERMILK QUALITY

Seven hundred and fifty cubic centimeter portions of raw skim milk were measured into each of six 1-liter Erlenmeyer flasks. Two of the flasks were heated in an Arnold steam sterilizer at 100° C. for 15 minutes; two others were heated in a water bath at 82° for the same length of time; the remaining two flasks were heated in a water bath at 63° for 30 minutes. After cooling and inoculating, one of each series was incubated at 37° and the others left at room temperature. The flasks that were heated to 63° only did not coagulate within 24 hours and in most cases two or three days passed before any coagulum was formed. When such coagulum was broken, it had a sandy texture and an extremely weak consistency. The mixture was composed of a large number of isolated particles of a medium coarseness floating in the serum. The slow coagulation, together with a pronounced foreign flavor, suggested that the sandy texture and weak consistency were due to resistant bacteria which survived the Pasteurization temperature and hindered the multiplication of the lactic culture. Many organisms were accordingly isolated, some of which are spore formers. The flavor of the product and an examination of the colonies on a milk-powder agar medium showed that some of those organisms produce acid, while others are of the peptonizing type. Moreover, a certain colloidal factor also may have operated; the importance of the chemical and physical factors has been very much overlooked in the past.

There was a marked improvement in the body of the product in the remaining flasks when the milk was heated to 100° C. as compared to the product made from milk heated to 82°. The probable reason for that difference is discussed in another section of this article.

The tendency of the product incubated at 37° to whey off and to increase in lump content was especially noticeable. A bad flavor found in all of the flasks, and more pronounced in the flasks heated to 82°, was due, probably, to the action of some spore formers which survived and were able to multiply rapidly enough at 37° to cause a change in the flavor.

The tendency to whey off at a rather high temperature seems to be mainly a physical phenomenon. The structure of the coagulum, in all probability, is similar to that of a sponge; its mass is penetrated, in all directions, by a network of very fine capillaries which

hold the original serum of the milk. The capacity of those capillaries to hold serum decreases with rising temperature. That has been shown by Wolf (*6, p. 524*) who measured the capillary rise of various liquids up to their critical temperatures. The capillary constants decreased gradually and became nil near the critical temperature, while the meniscus disappeared and the surface of the liquid inside as well as outside of the capillary tube became horizontal. Because of the faster rate of acid production at that temperature, milk coagulates much faster at 37° C. than at room temperature. This rapid coagulation has a tendency to decrease the absorptive capacity of the coagulum, because capillary movement in very fine tubes is extremely slow due to the enormous friction inside such tubes. In order to make that point clearer the process of coagulation may be compared to that of crystallization. Both processes deal with particles of matter which come together; the main difference is in the magnitude of the particles. It is a well known fact that when crystallization takes place quickly the crystals formed contain only a small quantity of impurities, while a slow coagulation, on the other hand, results in the formation of impure crystals. The impurities, in the present case, consist of the serum and the salts dissolved in it. Throughout this study the beneficial effect of slow coagulation was observed. In all cases, when coagulation took place, it resulted in an improvement in the body.

Table 2 shows the effect of heat and incubation temperature on the quality of buttermilk, while Table 3 records the rate of acid production at ordinary and warmer temperatures.

An examination of Table 3 shows that acid production proceeds much faster at 37° C. than at room temperature, and that milk coagulates a few hours earlier at 37° than at room temperature. It reveals, moreover, the very interesting fact that, after coagulation takes place, the rate of acid production at 37° is reduced and that at room temperature it rises rapidly, so that the total amount of acid produced at room temperature finally exceeds that produced at 37°.

On the other hand, Loeb (*10*) has shown that the solubility of casein-acid salts is brought about by the forces resulting from Donnan's equilibrium. He observed that the solution of casein chloride particles in hydrochloric acid was preceded by an increase in swelling and he was able to measure that increase. He recorded, furthermore, a maximum solubility and also a maximum swelling at about P_H 2.0 of the outside solution. The higher acidity at room temperature therefore may be partly responsible for the softer curd and the fewer lumps observed at that temperature.

TABLE 2.—*Effect of heat and incubation temperatures on the quality of buttermilk*

Flask No.	Temperature at which heated	Temperature at which incubated (° C.)	Quantity of undesirable lumps (mgm.)		Viscosity in seconds	
			Series 1	Series 2	Series 1	Series 2
	° C.					
1	63	Room temperature	(a)	(a)	(a)	(a)
2	63	37°	(a)	(a)	(a)	(a)
3	82	Room temperature	1,532	1,231	32.2	34.4
4	82	37°	4,557	4,875	----------	86.0
5	100	Room temperature	470	391	23.3	25.2
6	100	37°	4,486	4,012	54.6	57.2

[a] Did not curdle within 24 hours.

TABLE 3.—*Rate of acid production in milk at different temperatures of incubation*

Number of hours between inoculation and taking of acidity record	Percentage acidity in form of lactic acid		Condition of sample
	Room temperature	37° C.	
9	0.210	0.250	Neither sample curdled.
12	.495	.675	Sample at 37° C. curdled.
16	.725	.770	Both samples curdled.
21	.760	.780	Do.
24	.830	.800	Do.

EFFECT OF SODIUM SALTS ON QUALITY OF BUTTERMILK

GENERAL EFFECT

The experiments to test the effect of sodium salts on the quality of buttermilk were performed in the manner described above with various sodium salts. The effect of the sodium as well as of the acid radicals was studied. The following general conclusions were drawn from these experiments:

1. All sodium salts studied, when added in the appropriate concentration, produced some improvement in the body of buttermilk.
2. The action of sodium salts is specific and represents the action of the sodium and of the acid radical.
3. When added in a certain concentration sodium salts alter the flavor. The extent of the change in flavor varies with the specific salt and the concentration in which it is added. Even tasteless salts altered the flavor of buttermilk. The concentration which produces the desired body has always been higher than that required to alter the flavor.

Titration experiments made as a part of this study, also point to the fact that at least some of the salts added to the milk increased the titratable acidity of the buttermilk. Table 4 illustrates some of the results obtained in the titration tests.

TABLE 4.—*Effect of sodium salts on the quality of artificial buttermilk*

Name of salt	Percentage added	Quantity of undesirable lumps (mgm.)	Viscosity in seconds	Percentage of acidity as lactic acid
Rochelle salt	0.0	506	21.5	.....
	.1	296	22.8	.....
	.2	147	22.0	.....
	.3	89	21.8	.....
	.4	69	20.4	.....
	.5	76	20.0	.....
Sodium chloride	0.0	106	33.6	0.623
	.1	49	24.6	.635
	.2	29	.....	.....
	.3	0	21.2	.650
	.4	0	18.4	.....
	.5	0	17.6	.655
Sodium nitrate	0.0	459	34.0	0.623
	.1	337	31.2	.....
	.2	291	28.4	.....
	.3	183	24.6	.685
	.4	107	22.3	.....
	.5	92	21.4	.706

Other sodium or potassium salts used in the test include the malates, the carbonates, the phosphates, the acetates, and the mucates. It was not deemed necessary to report the detail of the figures obtained, as an exact idea of the relative efficiency of some of those salts may be had from a study of Tables 5 and 6, which show the separate action of sodium and that of a few acid radicals.

SPECIFIC EFFECT OF RADICALS

The statement has already been made that the effect of a sodium salt represents the combined effect of the sodium and of the acid radical. That statement is based on experiments of which the following is an illustration:

To several flasks, each containing 750 c. c. of skim milk, varying portions of different salts were added. The salt portions were such that the same weight of sodium was introduced into each. All of the flasks received similar treatments except that different flasks received different acid radicals. Any variations in the resulting effects may be ascribed therefore to that source. The results are summarized in Table 5.

TABLE 5.—*Effect of the acid radicals of salts upon the body of artificial buttermilk*

[The quantities added correspond to 0.5 gm. of sodium per flask]

Name of salt	Number of grams of salt per flask	Quantity of undesirable lumps (mgm.)	Viscosity in seconds
Sodium mucate	2.7838	457	35.0
Disodium phosphate	3.8939	494	30.8
Trisodium citrate	2.5881	291	24.6
Sodium acetate	2.9565	138	19.4
Sodium chloride	1.2717	198	18.0
Disodium sulphate	1.5435	144	16.2

In the experiment reported in Table 6 monosodium salts were added so that the 0.23 gm. of sodium added to each flask corresponded to equal concentrations of the acid radicals.

TABLE 6.—*Effect of acid radicals on the body of artificial buttermilk*

[The quantities added correspond to 0.23 gm. of sodium per flask]

Name of salt	Moles per flask	Quantity of undesirable lumps (mgm.)	Viscosity in seconds
Sodium chloride	0.01	11	23.9
Sodium acetate	.01	15	20.4
Monosodium phosphate	.01	102	.....

In order to observe the effect of sodium in the experiment, salts of a polybasic acid like phosphoric acid containing equal fractions of the phosphoric radical were used. Thus the flask receiving the disodium salt automatically received twice as much sodium as the flask receiving, in the monosodium salt, an equal concentration of the phosphoric radical. The acid radicals of both salts may be

considered equivalent qualitatively, just as they are quantitatively, for Loeb (*10*) has shown that divalent and polyvalent radicals of acids like phosphoric, citric, and tartaric, react with proteins very much as do monovalent radicals. Table 7 illustrates the results obtained in the experiments with monosodium and disodium phosphate.

TABLE 7.—*Effect of sodium on the body of artificial buttermilk*

Name of salt	Moles per flask	Quantity of undesirable lumps (mgm.)	Viscosity in seconds
Monosodium phosphate	0.01	168.0	18.4
Disodium phosphate	.01	104.0	19.2

A careful examination of the tables recording the effect of sodium salts on the quality of buttermilk shows clearly the beneficial effect of sodium salts in reducing the lumpiness and the viscosity of buttermilk. In fact, the body may be weakened at will and may finally reach a point where the formation of a coagulum ceases, resulting in a product slightly thicker than milk but having the characteristic flavor of buttermilk.

It is evident also that sodium salts have individual action resulting from their sodium and acid constituents. It is possible to divide the different salts studied into groups having a similar, but not identical action. The chlorides, the acetates, and the sulphates, for instance, give a very soft, jellylike coagulum, reduce the tendency to whey off, and weaken the body of buttermilk extensively. The phosphates and the mucates increase the cohesion of the particles and have little effect in reducing lumpiness in the lower concentrations. While the citrates, tartrates, and malates hold a middle position, the carbonates form a class apart because of the very weak, gassy curd which they produce.

It must be understood that this classification of sodium salts is correct only in a general way, and that the individual salts within the same group vary widely in their action and the characteristic curd they produce.

THE CHEMICAL PROCESSES INVOLVED WHEN A SODIUM SALT IS ADDED TO MILK AND WHEN MILK IS HEATED

ACTION OF SODIUM SALTS

It is not the object of this work to present a general discussion of the views held by different investigators regarding the chemical and physicochemical constitution of milk. The view held by Van Slyke and Bosworth (*13*) and prevalent in this country is that casein in milk is in the form of tetracalcium or tricalcium caseinate. When a sodium salt is added to normal milk, a reaction of the following type probably takes place: Calcium caseinate + sodium chloride $\rightleftarrows$ calcium sodium caseinate + calcium chloride.

The addition of $CaCl_2$ or of NaCl makes the reaction proceed in one direction or the other. Thus enough $CaCl_2$ may be added to precipitate the casein as calcium caseinate, while an excess of NaCl

results in the formation of the very soluble sodium caseinate or of calcium sodium caseinate, and an increase in soluble calcium which is replaced by sodium (*3*, *13*).

Laqueur and Sackur (*7*) explain the increase in viscosity observed when sodium hydroxide is added to a casein solution, by the formation of the highly dissociated sodium caseinate. Beyond a certain point, a further addition of the alkali causes a drop in viscosity. Laqueur and Sackur attribute that drop in viscosity to the depressing effect of the sodium on the dissociation of sodium caseinate. Pauli (*11*), accepting their view, goes further, advancing the hypothesis that each protein ion is hydrated; that is, each individual protein ion is surrounded by a shell of water, while the molecules are not hydrated. According to Pauli, hydration of the ions accounts for the higher viscosity of the more dissociated salts.

The studies made by various investigators thus indicate that a sodium salt added to normal milk results in the formation of the soluble and highly dissociated sodium caseinate or of a calcium-sodium caseinate characterized, according to Bosworth (*3*), by the softness and the fine texture of their curds. This highly dissociated sodium caseinate results in a more uniform coagulum which, when broken, produces a smooth, lump-free body. Moreover, sodium caseinate, being very soluble, does not coagulate readily; a softer curd and an increased capacity to absorb water therefore result.

In addition to their beneficial effects on milk by the formation of sodium caseinate, sodium salts contribute to some extent in at least four other ways to an improvement in the body of buttermilk.

They increase the solvent action of the serum. Lindet (*8*, *9*) has demonstrated that the casein of milk is soluble in the serum constituents, and has shown that the presence of the so-called "lactalbumin" in milk is due entirely to the solvent action of the serum on milk caseins. An addition of sodium citrate to milk in the concentration of 4 parts per 1,000 increased its soluble proteins from 6.12 gm. to 8.94 gm. per liter. Consequently, it is very likely that some of the added salts exert a solvent action, thus increasing the softness of the curd. A simple calculation shows that a very small amount of sodium is sufficient to replace even the whole calcium of the caseinate molecule; the relatively large excess of sodium salt added is necessary, however, to make the reversible reaction shown above proceed in the desired direction; and when the salt used is capable of dissolving casein, that excess undoubtedly exerts a marked solvent action on the curd. Table 8 shows the results obtained by Lindet (*8*) with an artificial serum of the indicated concentration.

TABLE 8.—*Solubility of casein in the constituents of the serum*

Constituent	Concentration (parts per 1,000)	Quantity of casein dissolved (gm. per liter)
Lactose	50.0	0.270
Sodium chloride	2.0	.605
Sodium citrate	.8	.945
Sodium phosphate	.5	1.636

Adding a salt to milk probably results in the formation of some free acid after coagulation. The free acid formed by increasing the hydrogen-ion concentration increases at the same time the solubility of the casein-acid salt, resulting in a softer curd. Different acids produce different solvent actions. Chick and Martin (*4*) have observed the formation of some free acid in solutions of pure protein-acid salts. They attribute it to hydrolysis of those compounds as follows:

$$\text{Protein} - \text{Hcl} \rightleftharpoons \text{Protein} + \text{H}^+ + \text{Cl}^-$$

While this may be true in the case of sour milk, it is necessary to assume previous union between casein and free acid liberated from the added salt. The possibility of such liberation is clear if it is understood that in addition to the casein-acid salt, sour milk contains several other salts in solution, some citric acid, and an increasing amount of lactic acid. A part of those acids is in combination with casein; the rest remain in the serum either in the free state or united to the bases present in the milk. Salts and acids of the serum, on the other hand, are in a state of dynamic equilibrium which may be represented, in the case of sodium chloride, as follows:

$$\underset{\text{lactic acid}}{\text{CH}_3.\text{CHOH.COOH}} + \text{NaCl} \rightleftharpoons \text{HCl} + \underset{\text{sodium lactate}}{\text{CH}_3.\text{CHOH.COONA}}$$

or expressing it in another form, as follows:

$$\text{Hcl} = \text{K}\frac{[\text{CH}_3.\text{CHOH.COOH}][\text{NaCl}]}{[\text{CH}_3.\text{CHOH.COONa}]}$$

It is thus shown that the amount of free acid formed varies directly with the amount of the salt added and the concentration of lactic acid. To such a change is attributed the alteration in the flavor of buttermilk even when a tasteless salt has been added to the milk.

The salts added as well as the free acid formed may affect the cohesion of particles. The strong cohesion that the addition of phosphates to milk causes in the resulting coagulum has already been pointed out. Loeb (*10*) found that acetic acid markedly decreases the cohesion between the particles of gelatin. This agrees with the present study which shows that the addition of acetates to milk results in the formation of a smooth curd relatively free from lumps.

Some of the salts added have a strong buffer action which, by slowing down the rate of increase in hydrogen-ion concentration, tends to produce a slow coagulation. It must be understood, nevertheless, that in a complex mixture like milk, this is a minor factor. Table 9 gives the buffer values of different salts in various concentrations as found by Loeb (*10*).

TABLE 9.—*The buffer action of salts on milk*

Name of salt	Molecular concentrations			
	0	M/64	M/16	M/4
Sodium chloride	3.0	3.00	3.00	3.00
Sodium sulphate	3.0	3.05	3.20	3.35
Monosodium phosphate	3.0	3.20	3.40	3.50
Monosodium tartrate	3.0	3.45	3.55	----
Monosodium citrate	3.0	3.60	3.75	----
Sodium acetate	3.0	4.60	----	----

ACTION OF HEAT

Several important changes in milk have been attributed to heat. Emphasis was laid in the past on the purely chemical ones, while the more recent investigations stress the changes that take place in milk as a colloidal system. Among the important chemical changes that take place are the precipitation of a part of the soluble milk proteins and also of the phosphates and the citrates in the form of saturated calcium salts, and the inactivation or destruction of milk enzymes. Most heated milks are coagulated with difficulty by rennet extract. How the above changes result in marked improvement in the body of buttermilk is hard to say. The bacterial factor, while very important, has been overestimated and is inadequate to explain the results obtained in this study. It is the writer's opinion that certain of the chemical changes promoted by heat are largely responsible for the improvement of the product, and that improvement by the addition of sodium salts and by heating are brought about largely through similar processes. As has been previously pointed out, the transformation of the sodium and calcium salts of casein to one another is a reversible change and proceeds in one direction or the other, depending on conditions. The change which takes place constantly in normal milk when sodium salt is added to it is illustrated by the formula,

$$\text{Calcium caseinate} + \text{sodium chloride} \rightleftarrows \text{sodium caseinate} + \text{calcium chloride}$$

The addition of sodium chloride causes the reaction to proceed to the right while the addition of calcium chloride has the opposite effect. The same conditions are brought about by the removal of calcium chloride and of sodium chloride, respectively. When milk is heated, some of the citrates and phosphates are precipitated as the saturated calcium salts and a reduction in the concentration of soluble calcium salts takes place. That throws the above reaction to the right. A study of the relation between the improvement observed at different temperatures in the present study, and the decrease in the concentration of soluble calcium salts at the same temperatures, as determined by other investigators, revealed that they proceed in the same direction; although, due to other effects of heat, it was found that the improvement proceeds faster. The changes referred to are mainly of a colloidal nature. They are not very well understood, but investigations made with other proteins show that they tend to decrease the stability of milk. Chick and Martin (*5*) report that a solution of egg white which has been denatured by heat, precipitates at a considerable range of hydrogen-ion concentration, but that precipitation takes place more slowly as the concentration becomes greater and also less. Here again, the beneficial effect of slow coagulation is observed.

MISCELLANEOUS EXPERIMENTS

Starch and gelatin were added to milk in different concentrations and the effect on the resulting buttermilk was studied. The results obtained show that gelatin has practically no effect on the lump content of buttermilk. When starch in concentrations ranging from

0.1 to 0.5 per cent is added to the milk before heating and the milk is incubated at room temperature, the resulting buttermilk tends to increase in lumpiness and viscosity. When starch in concentrations of 0.2 per cent or above is added under the same conditions, the resulting buttermilk tends to have a starchy flavor.

Because of the sensitiveness of buttermilk to foreign flavors an attempt was made to improve its body by purely physical means. The product was run through a homogenizer at pressures of 2,500, 4,000, and 5,000 pounds, respectively. The results were surprisingly disappointing. Instead of the smooth, creamy, lump-free buttermilk expected, this test resulted in a liquid that separated on standing for a few minutes into two layers. The lower layer consisted of most of the serum from the buttermilk, and the top layer of a spongy, light, dry curd. Homogenization, while reducing the size of particles, failed to reduce the curd to colloidal dimensions; the pressure and the mechanical breakage of the capillaries resulting from homogenization served to express most of the serum from the curd.

Homogenizing the milk at different temperatures and pressures was also tried, but no improvement resulted. The data secured from homogenizing milk are recorded in Table 10.

TABLE 10.—*Effect of homogenizing milk before souring on the quality of artificial buttermilk*

Flask No.	Treatment	Homogenizing temperature (°C.)	Quantity of undesirable lumps (mg.)	Viscosity in seconds
1	Homogenized	9	139	28.2
2	do	38	141	28.2
3	do	38	136	27.4
4	Not homogenized		130	27.3
5	do		159	28.0

CONCLUSIONS

The influence of bacterial factors on the quality of artificial buttermilk has been emphasized to the exclusion of chemical factors. This study was conducted in order to determine whether or not the quality of buttermilk can be influenced by chemical means.

It was found that quality may be improved by the addition of various sodium salts to the milk before souring, but that such treatment resulted in altering the flavor of the product.

Heating milk at 100° C. produced marked improvement over heating at 82°. This difference, however, was not due entirely to the elimination of harmful microorganisms.

An attempt to study the mechanism of the chemical changes involved in making buttermilk led to evidences suggesting that adding a sodium salt to milk and heating it would improve the quality of the resulting buttermilk by similar processes. The tendency of artificial buttermilk to whey off when incubated at rather high temperatures was found to be due largely to physical forces.

Homogenizing artificial buttermilk resulted in the separation of serum; homogenizing milk before souring produced no beneficial effects.

Under the conditions of the experiments discussed in this study, substances like gelatin and starch produced no improvement in quality of buttermilk.

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NET-ENERGY VALUES OF CORN SILAGE, SOY-BEAN HAY, ALFALFA HAY, AND OATS[1]

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INTRODUCTION

The object of the respiration calorimetric experiments herein recorded was to gain a wider knowledge of the net-energy values of feeds for cattle.

The experimental subjects were three Aberdeen Angus steers each weighing about 800 pounds. Steer No. 254 was calved on February 8, No. 36 on October 10, and No. 47 on August 29—all in the year 1924—and the experimental program was conducted between October, 1926, and May, 1927. The ages of these steers, therefore, during the experiments were as follows: No. 254, 32 months and 19 days to 36 months and 18 days; No. 36, 27 months and 16 days to 29 months and 29 days; and No. 47, 26 months and 1 day to 32 months and 8 days. During the five months preceding these experiments the steers had been on good pasture without grain feed; they were, therefore, in good flesh; that is, about half fat.

The plan of the experiments contemplated (1) the determination of the fasting heat production of the experimental subjects as measures of the maintenance requirement of energy, and (2) the complete accounting for the energy of the rations used, each at two levels of intake, maintenance and production. The schedule of these experiments comprises Table 1.

FEEDS AND RATIONS

During periods 1, 7, 9, and 14 the subjects received no feed; and during the remaining 10 periods they received corn silage, soy-bean hay, or alfalfa hay, either alone or with a grain supplement.

The steers, however, refused to eat without waste the rations which the writers wished to study in quantities sufficient for the production of any considerable body increase. In the course of the season's experiments, many changes of plan were imposed by this action of the experimental subjects; the contemplated periods on full feed were found to be impracticable; corn stover, which had been included in the list of feeds to be studied, had to be dropped from the experimental program; and the amounts of the rations finally fed, as determined by the willingness of the steers to eat them without waste, were in general about such as were required to maintain energy equilibrium.

The planes of nutrition in the 10 feeding periods are shown by the balances of energy in Table 1. The amount of the smallest ration fed in period 3 was arbitrarily fixed at one-half that eaten in period 2. Otherwise the steers received about as much as they would take without any waste.

[1] Received for publication Mar. 2, 1927; issued June, 1927.

TABLE 1.—*Experimental periods, rations, and live weights of animals, experiment No. 237*

Period No.	Steer No.	Preliminary feeding period on experimental ration	Total digestion periods	Calorimeter periods	Daily rations				Balance of energy	Average live weight
					Roughage		Concentrate			
					Kind	Amount fed daily	Kind	Amount fed daily		
						Kgm.		*Kgm.*	*Cals.*	*Kgm.*
1	254	Fasting		Oct. 27–29					Fast.	357.2
2	47	Oct. 30–Nov. 4	Nov. 5–19	Nov. 17–19	Corn silage	12.0	Cottonseed meal	0.450	1,490.4	359.8
3	254	Nov. 15–20	Nov. 21–Dec. 3	Dec. 1–3	do	6.0	do	.225	−1,866.4	345.3
4	47	Nov. 22–29	Nov. 30–Dec. 17	Dec. 15–17	do	12.0			434.0	361.5
5	254	Dec. 10–13	Dec. 14–Dec. 31	Dec. 29–31	Soy-bean hay	3.8			−1,392.3	347.0
6	47	Dec. 28–Jan. 2	Jan. 3–Jan. 14	Jan. 12–14	do	4.5			103.7	357.4
7	36	Fasting		Jan. 26–29					Fast.	334.0
8	254	Jan. 27–Jan. 31		Feb. 9–11	Corn silage	3.9	Oats	2,000	389.8	339.7
9	254	Fasting	Feb. 1–19	Feb. 23–26					Fast.	333.7
10	36	Feb. 11–21		Mar. 9–11	Alfalfa hay	4.6			−923.0	329.5
11	47	Mar. 10–14	Feb. 22–Mar. 11	Mar. 23–25	do	5.0			−753.1	355.0
12	36	Mar. 14–21	Mar. 15–26	Apr. 6–8	do	2.0	Oats	2.000	327.5	318.5
13	47	Mar. 27–Apr. 4	Mar. 22–Apr. 8	Apr. 20–22	do	2.13	do	2.130	655.9	347.0
14	47	Fasting	Apr. 5–22	May 4–7					Fast.	344.5

The difficulties encountered and the compromises necessitated in the feeding of the steers were due to the fact that whereas the method of experimentation required that the feed be consumed without waste, it is customary for cattle receiving considerable amounts of roughage to do some picking and choosing, and the rations which the writers wished to study were less palatable than those on which the steers had been reared.

In periods 2 and 3 a very small amount of cottonseed meal was fed with the corn silage in an effort to stimulate feed consumption, to avoid nitrogen loss, and to prevent an anticipated laxative tendency of the silage. The effort to stimulate feed consumption was not successful; the nitrogen balance was practically an equilibrium in both periods (Table 3); and corn silage by itself, in the largest quantity fed, 12 kgm. (26.2 pounds), proved not to be definitely laxative.

In periods 5 and 6 soy-bean hay was fed alone. In an effort to obtain a highly palatable hay without woody stems, which the animals would reject, the beans were cut when in bloom—somewhat earlier than is customary—but even so, and in spite of the fact that the hay was very successfully cured, the animals could not be induced to eat any considerable quantity. In period 5 steer No. 254 lost 1,392 Calories per day—a comparatively small amount—while in period 6 steer No. 47 was almost exactly in energy balance.

In periods 8, 12, and 13 ground oats were fed with either corn silage or alfalfa hay, and thus it became possible, by a comparison of the results with those obtained with the roughages alone, to determine the net-energy value of the oats.

In periods 10 and 11 alfalfa hay was fed alone. The hay was of good quality, but the animals would not eat quite enough of it, by itself, to maintain energy equilibrium.

The net-energy values determined in this series of experiments, therefore, apply not to production but much more nearly—and without considerable error—to maintenance.

EXPERIMENTAL PROCEDURE

The duration of the preliminary feeding on the kind of feed to be used in the subsequent experimental period was in no case less than 10 days, while the length of the preliminary period on the particular amount of feed to be used in the subsequent experiment was as indicated in Table 1, column 3; the length of digestion periods (normally 18 days, but sometimes necessarily abbreviated) is given in column 4; and the duration of the periods of continuous calorimeter experimentation was as indicated in column 5, all dates being inclusive.

The calorimeter periods in which the animals received feed were all 3 days in length, while the fasting calorimeter periods were 3 or 4 days in length—3 in period 1 and 4 in periods 7, 9, and 14.

In the preparation of the steers for the determination of the fasting katabolism roughage was withheld for a day, and grain for the latter half of the same day. Then each steer was given two half-pound doses of physic, two hours apart, the physic being composed of the following components in the proportions indicated: Sodium sulphate 12 ounces, capsicum 2 ounces, ginger 2 ounces, and gamboge 1 ounce. This physic was administered in the morning of a given day and the measurement of the heat production of fast (except in period 1) began on the second morning afterward, the first day of

measurement, therefore, being the third day of fast. In period 1 the first day of the heat measurement was the eighth day of fast.

Salt was fed in all feeding periods to the amount of 30 gm. (15 gm. per feed) per day. An effort was made to have the fasting steers take a nutrient salt mixture to prevent loss of salts from the body and to stimulate water drinking, but the steers did not care for it. Fasting cattle have no appetite for salt, and comparatively little desire for water.

All feeds were sampled for analysis in the usual manner at the time they were weighed out for feeding. In the case of corn silage this involved daily sampling, two 2-quart bottles being taken each day. These daily portions were kept in a cold-storage room, at a temperature below 0° F., and were combined and sampled for analysis at the end of the experimental period. The feeds, except the corn silage, were analyzed from the fresh condition.

The analysis of the silage, except for the determination of nitrogen and carbon, was made on material dried in a steam-heated air oven at a temperature of about 60° C. Nitrogen was determined in the fresh material. Carbon was determined in a combustion furnace on fresh material, cut up by a draw-cut meat chopper, and also in the dried material. The energy determination on the dried material was corrected for the loss of carbon in drying by adding 9.4 Calories for each gram of carbon lost, on the assumption that this loss was carbohydrate.

The feces were sampled daily, the combined aliquots being kept at a temperature below 0° F., without preservative, until the end of the current experimental period, when they were sampled for analysis.

The feces were analyzed from the air-oven-dried material, except that nitrogen and carbon were also determined in the fresh product; dry matter was directly determined by means of the vacuum desiccator; and energy was determined in the vacuum-dried material. The energy as determined was corrected for the slight loss of carbon and nitrogen during drying.

Urines were sampled daily and preserved at about 34° F., with the addition of chloroform.

EXPERIMENTAL DATA

The digestibility of the rations is shown in Table 2. These data are not used in the determination of net energy, but are included because they are available, and for such interest as they may have for students and others who use such values.

There are a number of appreciable differences between these coefficients of digestibility and the average values compiled by Henry and Morrison,[2] though most of these lie within the range of normal variation, as affected by the composition of the feeds and the individuality of the animal used as the experimental subject. The only notable difference is in the digestibility of the ether extract of the alfalfa hay, in regard to which the very low values found may be due to the low planes of nutrition prevailing and to the ether extract of the feces being largely of metabolic origin instead of being derived solely from the feed, as implied by the method of computation of digestibility.

The balances of matter and energy are recorded in Table 3. The computed heat production is derived by computation from the income

[2] HENRY, W. A., and MORRISON, F. B. FEEDS AND FEEDING. Ed. 18, unabridged, 770 p., illus. Madison, Wis. 1923.

and outgo of carbon, nitrogen, and energy; the observed heat production is the heat directly measured; and the balance of energy is the metabolizable energy minus the observed heat production.

TABLE 2.—*Digestibility of rations, experiment No. 237*

Period No.	Animal No.	Feeds and other items	Dry matter	Organic matter	Crude protein	Crude fiber	N-free extract	Ether extract	Carbon	Energy
			Grams	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Cals.*
2	47	Salt	30.0							
		Silage	3,607.0	3,411.8	294.0	835.7	2,190.5	91.6	1,702.0	16,473.0
		Cottonseed meal	411.7	381.3	191.9	39.5	120.0	29.9	197.5	2,051.8
		Total feed	4,049.0	3,793.1	485.9	875.2	2,310.5	121.5	1,899.5	18,524.8
		Feces	1,164.0	1,031.4	187.6	307.2	518.6	18.0	554.6	5,477.1
		Percentage digestibility	71.3	72.8	61.4	64.9	77.6	85.2	70.8	70.4
3	254	Salt	30.0							
		Silage	1,794.0	1,693.3	135.8	431.6	1,080.0	45.9	861.5	8,412.2
		Cottonseed meal	205.8	190.8	96.0	21.6	58.2	15.0	99.1	1,033.1
		Total feed	2,029.8	1,884.0	231.8	453.2	1,138.2	60.9	960.6	9,445.3
		Feces	562.9	488.7	95.8	140.5	242.5	9.9	266.1	2,727.4
		Percentage digestibility	72.3	74.1	58.7	69.0	78.7	83.8	72.3	71.1
4	47	Salt	30.0							
		Silage	3,370.6	3,197.3	275.3	785.3	2,047.5	89.2	1,610.3	15,526.1
		Feces	1,004.0	909.5	137.7	276.7	481.5	13.7	500.3	4,921.7
		Percentage digestibility	70.5	71.6	50.0	64.8	76.5	84.6	68.9	68.3
5	254	Salt	30.0							
		Soy-bean hay	3,337.5	3,054.2	664.5	1,050.7	1,275.0	64.0	1,537.9	15,099.5
		Feces	1,261.6	1,082.7	165.7	505.1	368.9	43.0	606.9	6,032.7
		Percentage digestibility	62.2	64.6	75.1	51.9	71.1	32.7	60.5	60.1
6	47	Salt	30.0							
		Soy-bean hay	3,979.4	3,653.6	772.1	1,252.5	1,561.2	67.9	1,837.8	18,128.0
		Feces	1,489.7	1,314.6	194.1	610.9	458.7	50.9	713.9	7,031.3
		Percentage digestibility	62.6	64.0	74.9	51.2	70.6	25.0	61.2	61.2
8	254	Salt	30.0							
		Silage	1,125.5	1,065.4	91.2	272.4	670.5	31.3	541.4	5,239.5
		Oats	2,260.2	2,175.1	307.3	267.1	1,494.6	106.1	1,067.1	10,756.6
		Total feed	3,415.7	3,240.5	398.5	539.5	2,165.1	137.4	1,608.5	15,996.1
		Feces	1,081.5	978.2	129.5	310.9	526.5	11.3	511.1	5,120.2
		Percentage digestibility	68.3	69.8	67.5	42.4	75.7	91.8	68.2	68.0
10	36	Salt	30.0							
		Alfalfa hay	4,016.2	3,672.9	641.4	1,343.8	1,625.7	62.0	1,862.3	18,159.8
		Feces	1,780.5	1,538.4	227.9	730.5	513.4	66.6	877.2	8,610.9
		Percentage digestibility	56.0	58.1	64.5	45.6	68.4		52.9	52.6
11	47	Salt	30.0							
		Alfalfa hay	4,426.9	4,038.6	720.0	1,465.3	1,783.5	69.8	2,067.5	20,428.0
		Feces	1,968.6	1,688.0	246.8	808.7	567.3	65.3	990.8	9,774.3
		Percentage digestibility	55.8	58.2	65.7	44.8	68.2	6.5	52.1	52.2
12	36	Salt	30.0							
		Alfalfa hay	1,782.2	1,639.3	265.8	612.6	734.4	26.6	826.2	8,123.8
		Oats	1,753.4	1,691.3	244.3	199.0	1,163.1	84.9	826.6	8,332.8
		Total feed	3,565.6	3,330.6	510.0	811.6	1,897.5	111.5	1,652.7	16,456.6
		Feces	1,312.1	1,162.4	169.7	470.6	491.0	31.0	627.8	6,247.7
		Percentage digestibility	63.2	65.1	66.7	42.0	74.1	72.2	62.0	62.0
13	47	Salt	30.0							
		Alfalfa hay	1,899.6	1,745.5	287.4	657.2	773.4	27.5	879.5	8,546.6
		Oats	1,866.7	1,797.4	259.8	217.6	1,231.0	89.0	876.6	8,798.2
		Total feed	3,796.3	3,542.9	547.2	874.8	2,004.4	116.5	1,756.1	17,344.8
		Feces	1,278.0	1,131.4	152.5	474.9	473.2	30.8	610.1	6,103.6
		Percentage digestibility	66.3	68.1	72.1	45.7	76.4	73.5	65.3	64.8

The results of the experiments are based on the directly determined heat production, the computed values being used simply as controls.

The fasting heat production, which constitutes the measure of the maintenance requirement of energy, is given in Table 4. This measurement as now taken is quite acceptable, but an effort is being made to improve it by determining the exact treatment necessary entirely to free the alimentary tract from feed residues, and by measuring directly instead of computing the surface area.

It will be noted that the fasting data used were derived from periods 7, 9, and 14. The results of period 1 were not used since these represented days of fast 8, 9, and 10, whereas it was found that the average of the results from days 3, 4, and 5, seemed to represent an approximately true state of fast, and were preferred as derived from the

shortest period acceptable for the purpose. In period 14 the electric power was off during part of the first calorimeter day and on this account the results of only the last two days, representing the fourth and fifth days of fast, were used.

TABLE 3.—*Balance of matter and energy per head and day, experiment No. 237*

PERIOD 2, STEER NO. 47

Item	Dry matter	Water	Nitrogen	Carbon	Energy
	Grams	*Grams*	*Grams*	*Grams*	*Cals.*
Income:					
Corn silage	3,607.0	8,385.0	47.0	1,702.0	16,473.0
Cottonseed meal	412.0	38.4	30.7	197.5	2,051.8
Water		4,055.0			
Total	4,019.0	12,478.4	77.7	1,899.5	18,524.8
Outgo:					
Feces	1,164.0	4,196.0	30.0	554.6	5,477.1
Urine	201.3	4,092.0	39.2	82.9	[a] 842.5
Methane	110.3			82.5	1,471.8
Carbon dioxide	3,936.2			1,073.4	
Water vapor		5,444.5			
Metabolizable:					
Income minus urine, feces, and methane					10,733.4
Body balances:					
Fat	103.6			79.3	984.2
Protein	51.0		8.5	26.8	[a] 227.4
Energy					[b] 1,490.4
Water		−1,254.1			
Computed heat production					[c] 9,521.8
Observed heat production					9,243.0

PERIOD 3, STEER NO. 254

Income:					
Corn silage	1,794.0	4,202.7	21.7	861.5	8,412.2
Cottonseed meal	205.8	19.2	15.4	99.1	1,033.1
Water		2,937.0			
Total	1,999.8	7,158.9	37.1	960.6	9,445.3
Outgo:					
Feces	562.9	1,666.0	15.3	266.1	2,727.4
Urine	166.3	4,137.0	30.8	55.6	[a] 428.6
Methane	78.5			58.8	1,047.5
Carbon dioxide	2,766.6			754.4	
Water vapor		3,306.0			
Metabolizable:					
Income minus urine, feces, and methane					5,241.8
Body balances:					
Fat	−190.7			−145.9	−1,811.7
Protein	−54.0		−9.0	−28.4	[a] −240.7
Energy					[b] −1,866.4
Water		−1,950.1			
Computed heat production					[c] 7,294.2
Observed heat production					7,108.2

PERIOD 4, STEER NO. 47

Income:					
Corn silage	3,370.6	8,656.0	44.0	1,610.3	15,526.1
Water		1,037.0			
Total	3,370.6	9,693.0	44.0	1,610.3	15,526.1
Outgo:					
Feces	1,004.0	4,467.0	22.0	500.3	4,921.7
Urine	194.7	3,533.0	19.6	59.2	[a] 558.9
Methane	98.7			73.8	1,317.1
Carbon dioxide	3,325.6			906.9	
Water vapor		4,793.3			
Metabolizable:					
Income minus urine, feces, and methane					8,728.4
Body balances:					
Fat	81.7			62.5	776.2
Protein	14.4		2.4	7.6	[a] 64.2
Energy					[b] 434.0
Water		−3,100.3			
Computed heat production					[c] 7,888.0
Observed heat production					8,290.4

[a] Corrected to nitrogen equilibrium.
[b] Metabolizable energy minus observed heat production.
[c] The computed heat is obtained by subtracting algebraically the gains of energy from the metabolizable.

TABLE 3.—*Balance of matter and energy per head and day, experiment No. 237*—Continued

PERIOD 5, STEER NO. 254

Item	Dry matter	Water	Nitrogen	Carbon	Energy
	Grams	*Grams*	*Grams*	*Grams*	*Cals.*
Income:					
Soy-bean hay	3,337.5	462.0	106.3	1,537.9	15,099.5
Water		11,013.0			
Total	3,337.5	11,475.0	106.3	1,537.9	15,099.5
Outgo:					
Feces	1,261.6	4,238.0	26.5	606.9	6,032.7
Urine	209.0	4,141.0	71.8	94.7	[a] 967.0
Methane	84.9			63.6	1,132.9
Carbon dioxide	3,176.7			866.3	
Water vapor		4,992.7			
Metabolizable:					
Income minus urine, feces, and methane					6,966.9
Body balances:					
Fat	−155.3			−118.8	−1,475.4
Protein	48.0		8.0	25.2	[a] 214.0
Energy					[b] 1,392.3
Water		1,896.7			
Computed heat production					[c] 8,228.3
Observed heat production					8,359.2

PERIOD 6, STEER NO. 47

Income:					
Soy-bean hay	3,979.4	521.3	123.5	1,837.8	18,128.0
Water		10,742.0			
Total	3,979.4	11,263.3	123.5	1,837.8	18,128.0
Outgo:					
Feces	1,489.7	5,577.0	31.1	713.9	7,031.3
Urine	232.7	5,134.0	83.4	104.5	[a] 1,060.0
Methane	103.2			77.2	1,377.1
Carbon dioxide	3,371.8			919.5	
Water vapor		5,103.6			
Metabolizable:					
Income minus urine, feces, and methane					8,659.6
Body balances:					
Fat	−7.5			−5.7	−71.3
Protein	54.0		9.0	28.4	[a] 240.7
Energy					[b] 103.7
Water		−4,551.3			
Computed heat production					[c] 8,490.2
Observed heat production					8,555.9

PERIOD 8, STEER NO. 254

Income:					
Corn silage	1,125.5	2,777.0	14.6	541.4	5,239.5
Oats	2,260.2	352.7	49.2	1,067.1	10,756.6
Water		6,145.0			
Total	3,385.7	9,274.7	63.8	1,608.5	15,996.1
Outgo:					
Feces	1,081.5	4,390.0	20.7	511.1	5,120.2
Urine	159.7	3,215.0	33.2	58.1	[a] 600.0
Methane	93.4			69.9	1,246.3
Carbon dioxide	3,480.4			949.1	
Water vapor		5,212.5			
Metabolizable:					
Income minus urine, feces, and methane					8,969.6
Body balances:					
Fat	−14.2			−10.9	−134.9
Protein	59.4		9.9	31.2	[a] 264.8
Energy					[b] 389.8
Water		−3,542.8			
Computed heat production					[c] 8,839.7
Observed heat production					8,579.8

[a] Corrected to nitrogen equilibrium.
[b] Metabolizable energy minus observed heat production.
[c] The computed heat is obtained by subtracting algebraically the gains of energy from the metabolizable.

TABLE 3.—*Balance of matter and energy per head and day, experiment No. 237*—Continued

PERIOD 10, STEER NO. 36

Item	Dry matter	Water	Nitrogen	Carbon	Energy
	Grams	*Grams*	*Grams*	*Grams*	*Cals.*
Income:					
Alfalfa hay	4,016.2	584.3	102.6	1,862.3	18,159.8
Water		10,707.0			
Total	4,016.2	11,291.3	102.6	1,862.3	18,159.8
Outgo:					
Feces	1,780.5	7,417.0	36.5	877.2	8,610.9
Urine	252.3	3,433.0	59.3	94.6	[a] 1,013.2
Methane	76.9			57.4	1,026.2
Carbon dioxide	3,297.6			899.3	
Water vapor		4,701.6			
Metabolizable:					
Income minus urine, feces, and methane					7,509.5
Body balances:					
Fat	−114.6			−87.7	−1,088.7
Protein	40.8		6.8	21.4	[a] 181.9
Energy					[b] −923.0
Water		−4,260.3			
Computed heat production					[c] 8,416.3
Observed heat production					8,432.5

PERIOD 11, STEER NO. 47

Income:					
Alfalfa hay	4,426.9	579.0	115.2	2,067.5	20,428.0
Water		11,920.0			
Total	4,426.9	12,499.0	115.2	2,067.5	20,428.0
Outgo:					
Feces	1,968.6	6,274.0	39.5	990.8	9,774.3
Urine	297.0	4,594.0	71.1	103.8	[a] 1,099.1
Methane	91.5			68.5	1,221.0
Carbon dioxide	3,611.9			985.0	
Water vapor		4,836.8			
Metabolizable:					
Income minus urine, feces, and methane					8,333.6
Body balances:					
Fat	−124.3			−95.1	−1,180.9
Protein	27.6		4.6	14.5	[a] 123.0
Energy					[b] −753.1
Water		−3,205.8			
Computed heat production					[c] 9,391.5
Observed heat production					9,086.7

PERIOD 12, STEER NO. 36

Income:					
Alfalfa hay	1,788.2	221.3	42.5	826.2	8,123.8
Oats	1,753.4	247.0	39.1	826.6	8,332.8
Water		10,082.0			
Total	3,541.6	10,550.3	81.6	1,652.8	16,456.6
Outgo:					
Feces	1,312.1	4,219.0	27.2	627.8	6,247.7
Urine	204.7	3,567.0	48.4	68.0	[a] 774.8
Methane	97.9			73.3	1,306.4
Carbon dioxide	3,188.0			869.4	
Water vapor		3,725.6			
Metabolizable:					
Income minus urine, feces, and methane					8,127.7
Body balances:					
Fat	−6.0			−4.6	−57.0
Protein	36.0		6.0	18.9	[a] 160.5
Energy					[b] 327.5
Water		−961.3			
Computed heat production					[c] 8,024.2
Observed heat production					7,800.2

[a] Corrected to nitrogen equilibrium.
[b] Metabolizable energy minus observed heat production.
[c] The computed heat is obtained by subtracting algebraically the gains of energy from the metabolizable.

TABLE 3.—*Balance of matter and energy per head and day, experiment No. 237*—Continued

PERIOD 13, STEER NO. 47

Item	Dry matter	Water	Nitrogen	Carbon	Energy
	Grams	*Grams*	*Grams*	*Grams*	*Gals.*
Income:					
Alfalfa hay	1,899.6	237.7	46.0	879.5	8,546.6
Oats	1,866.7	265.0	41.6	876.6	8,798.2
Water		10,880.0			
Total	3,766.3	11,382.7	87.6	1,756.1	17,344.8
Outgo:					
Feces	1,278.0	3,755.7	24.4	610.1	6,103.6
Urine	203.7	3,537.7	54.6	75.0	[a] 845.0
Methane	103.1			77.1	1,375.8
Carbon dioxide	3,403.7			928.2	
Water vapor		3,776.5			
Metabolizable:					
Income minus urine, feces, and methane					9,020.4
Body balances:					
Fat	50.5			38.6	479.8
Protein	51.6		8.6	27.1	[a] 230.0
Energy					[b] 655.9
Water		312.8			
Computed heat production					[c] 8,310.6
Observed heat production					8,364.5

[a] Corrected to nitrogen equilibrium.
[b] Metabolizable energy minus observed heat production.
[c] The computed heat is obtained by subtracting algebraically the gains of energy from the metabolizable.

TABLE 4.—*Daily fasting katabolism of steers, experiment No. 237*

Period No.	Steer No.	Day of fast	Body surface [a]	Heat production: Per head	Heat production: Per square meter of body surface
			Sq. m.	*Cals.*	*Cals.*
7	36	Third	4.15	6,465.6	1,558.0
		Fourth	4.13	5,920.1	1,433.4
		Fifth	4.11	5,830.5	1,418.6
		Average		6,072.1	1,470.0
9	254	Third	4.19	6,516.8	1,555.3
		Fourth	4.17	6,323.6	1,516.5
		Fifth	4.15	6,137.2	1,478.8
		Average		6,325.9	1,516.9
14	47	Fourth	4.26	6,663.8	1,564.3
		Fifth	4.24	6,403.2	1,510.2
		Average		6,533.5	1,537.3

[a] Computed by Moulton's formula, $0.1186\ W^{\frac{2}{3}}$, in which W, the empty live weight, is the actual live weight times 0.9. See MOULTON, C. R. UNITS OF REFERENCE FOR BASAL METABOLISM AND THEIR INTERRELATIONS. Jour. Biol. Chem. 24: 299-320, illus. 1916.

The final results of the series of experiments are stated in Table 5. The net energy for maintenance, per head, was computed from the fasting heat production per square meter of body surface (Table 4) by correction for the difference between the weight of the animal during the fasting and the feeding experiments (Table 1).

The data regarding heat production were as directly determined (Table 3); the total heat increments were derived by subtracting the

net energy requirements (per head) for maintenance from the corresponding figures for heat production; and the heat increments per kilogram of dry matter of feed were obtained by dividing the total heat increments by the kilograms of dry matter of feed eaten (Table 5).

The metabolizable energy per kilogram of dry matter was computed from the total metabolizable energy, as in Table 3, and the amount of dry matter of feed eaten; the net energy (for maintenance) per kilogram of dry matter is the similar figure for metabolizable energy minus the heat increment; and the utilization of the metabolizable energy is the percentage of the metabolizable energy which is net.

TABLE 5.—*Computation of net-energy values for maintenance, experiment No. 237*

Period No.	Animal No.	Dry matter (in kilograms) of feeds eaten	Total dry matter of feeds eaten	Live weight	Body surface	Net energy for maintenance		Heat production	Heat increment		Metabolizable energy per kilogram dry matter	Net energy per kilogram dry matter	Utilization of metabolizable energy
						Per square meter body surface	Per head		Total	Per kilogram dry matter of feed			
			Kgm.	*Kgm.*	*Sq. m.*	*Cals.*	*Cals.*	*Cals.*	*Cals.*	*Cals.*	*Cals.*	*Cals.*	*Per cent*
2	47	Silage, 3.607 Cottonseed meal, 0.412	4.019	359.8	4.40	1,537.3	6,764.1	9,419.4	2,655.3	661	2,671	2,010	75.3
3	254	Silage, 1.794 Cottonseed meal, 0.206	2.000	345.3	4.29	1516.9	6,507.5	7,129.7	622.2	311	2,621	2,310	88.1
2	47	Silage [a]									2,651	1,959	73.9
3	254	do.[a]									2,596	2,294	88.4
4	47	do	3.371	361.5	4.41	1,537.3	6,779.5	8,434.2	1,654.7	491	2,589	2,008	81.0
5	254	Soy bean hay	3.338	347.0	4.30	1,516.9	6,522.7	8,474.4	1,951.7	585	2,087	1,502	72.0
6	47	do	3.979	357.4	4.38	1,537.3	6,733.4	8,673.0	1,939.6	487	2,176	1,680	77.6
8	254	Silage, 1.126 Oats, 2.260	3.386	339.7	4.24	1,516.9	6,431.7	8,634.3	2,202.6	651	2,649	1,998	75.4
10	36	Alfalfa hay	4.016	329.5	4.16	1,470.0	6,115.2	8,515.7	2,400.5	598	1,870	1,272	68.0
11	47	do	4.427	355.0	4.36	1,537.3	6,702.6	9,159.6	2,457.0	555	1,882	1,327	70.5
12	36	Alfalfa hay, 1.788 Oats, 1.753	3.541	318.5	4.07	1,470.0	5,982.9	7,937.5	1,954.6	552	2,295	1,743	75.9
13	47	Alfalfa hay, 1.900 Oats, 1.867	3.767	347.0	4.30	1,537.3	6,610.4	8,486.9	1,876.5	498	2,395	1,897	79.2
12	36	Oats [a]							885.4	505	2,729	2,224	81.5
13	47	do.[a]							822.0	440	2,916	2,476	84.9

[a] See text for method of computation.

The results of the first two feeding periods, 2 and 3, in which a small proportion of cottonseed meal was fed, were used for the computation of the net-energy value (for maintenance) of corn silage by employing Kellner and Köhler's production value for cottonseed meal, as quoted by Armsby,[3] increased by the use of the ratio (1:0.761), determined at this institute,[4] between the utilization of energy for maintenance and production. While the basis of this computation is imperfect, the average of the two results (2,126.5 calories) is only about 1 per cent greater than the more directly determined value (2,098 Calories) found in period 4. It is therefore of little moment whether the three values for silage are averaged, or whether the third figure (2,098 Calories) is accepted. The result of period 2 (1,959 Calories) agrees fairly well with the more directly determined value (2,098 Calories) found in period 4. The relatively low heat increment and high net-energy values obtained in period 3

[3] ARMSBY, H. P. THE NUTRITION OF FARM ANIMALS. p. 660. New York. 1917.

[4] FORBES, E. B., FRIES, J. A., BRAMAN, W. W., and KRISS, M. THE RELATIVE UTILIZATION OF FEED ENERGY FOR MAINTENANCE, BODY INCREASE, AND MILK PRODUCTION OF CATTLE. Jour. Agr. Research 33: 483–492. 1926.

are quite likely due to the very low plane of nutrition, and can hardly be accepted as applying to maintenance. All things considered, it seems best to regard the directly determined figure (2,098 Calories) as the provisional net-energy value of corn silage, for maintenance.

The two values for net energy (for maintenance) of soy-bean hay (1,502 and 1,689 Calories) agree only fairly well, but are provisionally acceptable.

The net-energy value of silage and oats, as determined in period 8, may be utilized to yield a net-energy value of either component of the ration by the use of the value for silage, as found in period 4, or the value for oats, as found in periods 12 and 13. Inasmuch, however, as values so determined would involve possible error through the comparison of results obtained with different experimental subjects, and inasmuch as the values computed by this imperfect procedure differ materially from the other values reported, derived from results in which there is no confusion of individuality of experimental subjects, no use is made of the results of period 8 at this time.

The values determined for alfalfa hay in periods 10 and 11 (1,272 and 1,327 Calories) agree fairly well, the larger being about 4 per cent in excess of the smaller; and the average may be accepted as the provisional net-energy value of alfalfa hay for maintenance.

By the use of these values and the results of periods 12 and 13, in which alfalfa and oats were fed, the values found for net-energy of oats, for maintenance, were 2,224 and 2,476 Calories per kilogram of dry matter. The higher of these values is about 11 per cent in excess of the lower—which leaves appreciable improvement to be accomplished in future determinations.

In connection with the net-energy values presented in Table 5, it is well to note that the duplicate determinations for soy-bean hay (periods 5 and 6), alfalfa hay (periods 10 and 11), alfalfa hay and oats (periods 12 and 13), and oats alone (computed by difference) were obtained on different animals, and that in each case steer No. 47 gave the higher value for net and metabolizable energy, and the lower value for heat increment.

It is also striking that the heat increments are much less affected by the kind of feed than are the metabolizable energy values. This accounts to a large extent for the markedly different percentages of utilization of the metabolizable energy in the last column of Table 5. These data show that the metabolizable energy of concentrates is much more largely utilizable than is that of roughages. Net energy, therefore, is much more significant and consistent than would be metabolizable energy as a measure of the energy value of a feed in nutrition.

SUMMARY

The following net-energy values, per kilogram of dry matter of feeds, for the maintenance of approximately 800-pound 2 to 3 year old beef steers are submitted, these values being determined by direct calorimetry, using the heat production during fast as the measure of the maintenance requirement of net energy: Corn silage, 2,098 Calories; soy-bean hay, 1,502 and 1,689 Calories; alfalfa hay, 1,272 and 1,327 Calories; and ground oats, 2,224 and 2,476 Calories.

The heat increments caused by the feeds were found to be much more nearly proportional to the dry matter than to the metabolizable

energy of the feed; and the percentages of utilization of the metabolizable energy were found to be much affected by the nature of the feed, especially as to whether a roughage or a concentrate; from which it follows that the net energy of a feed is much more significant and consistent than is metabolizable energy (feed minus excreta) as a measure of the energy value of a feed in nutrition.

The maintenance requirements of net energy of the three steers used as subjects were 1,470, 1,517, and 1,537 Calories, respectively, per square meter of body surface, and 1,818, 1,896, and 1,896 Calories, respectively, per 100 kgm. (826, 859, and 859 Calories per 100 pounds) of live weight.

○